AECV93-R7

OLDMAN RIVER DAM: MERCURY IN FISH

INTERIM REPORT 1991

Alberta
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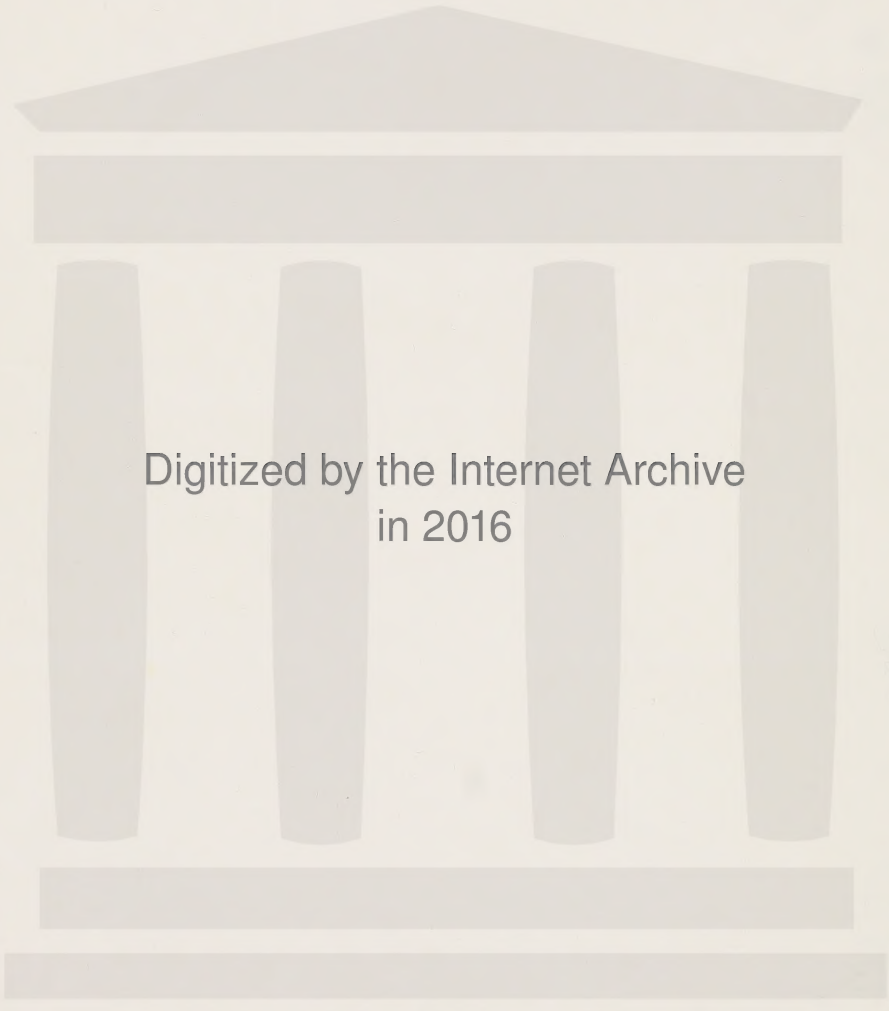

OLDMAN RIVER DAM: MERCURY IN FISH

INTERIM REPORT 1991

by

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1.0 SUMMARY

Fish were collected during 1991 for analysis of mercury in muscle tissue from two sites on the Oldman River Dam Reservoir, two sites downstream of the reservoir on the Oldman River, one upstream site on the Oldman River, and one upstream site on the Crowsnest River. The species of fish collected were mountain whitefish, rainbow trout, bull trout, brown trout, white sucker, longnose sucker, northern pike and burbot. Total mercury concentration in muscle tissue was assayed in 409 fish and organic mercury concentration in 123 fish. Based on these collections, it was noted that:

i) bull trout, white sucker and longnose sucker carried larger mercury concentrations than the other species,

ii) mean mercury concentrations were always <0.5 mg kg^{-1}, regardless of species or site,

iii) organic mercury accounted for approximately 95% of total mercury,

iv) total mercury concentrations in these 1991 collections were within the bounds of total mercury concentrations estimated for the same species prior to the dam's being constructed.

It was concluded that mercury residues in fish from the reservoir and surrounding rivers posed no health threat to human consumers of fish.

2.0 BACKGROUND

In 1991, the Alberta Environmental Centre (AEC) initiated a five year research project on mercury in fish inhabiting the newly formed Oldman River Dam Reservoir and rivers within the Oldman River basin. The project, conceived in association with three other government departments (Alberta Public Works, Supply and Services, Alberta Environment, and Alberta Forestry, Lands and Wildlife), formed part of a much larger fisheries mitigation strategy for the basin (Alberta Public Works, Supply and Services, 1990). This strategy requires that there be no net loss of fish habitat or recreational fishing opportunities, consistent with the government's policy of sustainable development.

The impetus for this project was the possibility that mercury concentrations in fish might increase after impoundment. Mercury, when found in fish, occurs primarily in an organic form (methyl mercury) that may affect the central nervous system of consumers. These effects, when fully manifested, induce a condition known as Minamata disease. The term Minamata refers to

Minamata Bay (Japan), the site where the disease was first diagnosed in fish consumers during the 1950's. Minamata disease does not appear to have been reported in Canada.

Increases in mercury in fish have been noted in several reservoirs in Canada and elsewhere (Green, 1990; Jackson, 1988; Bodaly *et al.*, 1984a; Abernathy and Cumbie, 1977), but has not been observed in Alberta (e.g. Alberta Environmental Centre 1989a). The mechanism for increased mercury uptake by fish appears to be based on enhanced activity of methylating bacteria and other microorganisms in freshly inundated soil (Cox *et al.*, 1979; Jackson, 1988). The rate of methylation and subsequent uptake by fish and other aquatic species depend on numerous factors, including redox potential of the sediment, binding of Hg^{2+} to sulphides, binding of organic mercury to manganese and iron hydoxides, microbiological activity, pH of the sediment and overlying water, mercury concentrations in water, temperature, and trophic conditions (Jackson, 1988; Berman and Bartha, 1986; Curtis, 1974; Hakanson, 1980). Since these factors may vary from reservoir to reservoir, the extent of mercury accumulation in fish is also variable. In the more serious cases where concentrations exceed the guideline of 0.5 mg kg^{-1} (muscle tissue), human consumption of fish has been limited or totally restricted.

This study has two objectives:

Primary - assess changes in the concentration of mercury in the muscle tissue of fish over a five year period in the Oldman Dam Reservoir, the Oldman River near the reservoir, and the Crowsnest River.

Secondary - conduct supplementary inventory studies of fish populations in the reservoir and surrounding rivers.

3.0 CONDUCT OF STUDY

3.1 Good Laboratory Practice

It is recognized that potential increases in the concentration of mercury in fish might constitute a significant human health problem. Hence, the principles of Good Laboratory Practice (GLP) were implemented during 1991, and will continue to be used throughout the duration (5 years) of the study. Compliance with GLP is intended to ensure the quality and integrity of data generated for safety testing and litigation.

Guidance for GLP (including animal care and use, and data and sample tracking) is outlined in Standard Operating Procedures (Aquatic Biology Branch, 1991). These procedures are consistent with those outlined by other agencies (Federal Register, 1983; National Health and Welfare, 1989). The AEC-approved protocol (#2440-DL2/P1), which is consistent with such standards, is appended to this report (Appendix A). Deviations from and amendments to the protocol are also included (Appendix B).

3.2 Project Team

A project team responsible for the execution of the protocol was formed and now includes the following staff (major duties in parenthesis):

J.W. Moore	Biological Sciences Division (Project Leader)
K.L. Smiley	Biological Sciences Division (Field Collection)
L.Z. Florence	Operations Division (Statistical Design and Analysis)
S. Wu	Physical and Engineering Sciences Division (Analytical methods)
D.S. Lucyk	Physical and Engineering Sciences Division (Laboratory Supervision and Analysis of Tissues)

All data and reports generated by this team are subject to the AEC review process.

3.3 Study Design and Sampling Methods

A description of the study design and sampling methods used in 1991 is outlined in Appendices A and B.

3.4 Mercury Analysis Method and Quality

Detailed descriptions of the methods used to determine total mercury and organic mercury are given in Appendices C and D, respectively. In Appendix C, both procedures of subsampling by snipping and subsampling from the homogenized tissue are documented. Although subsampling by the snipping procedure is simple, rapid and inexpensive, it may be subject to sampling error. On the other hand, subsampling from homogenized tissue has much less risk of sampling error, but is tedious and may be subject to high bias due to process contamination and moisture loss. For the purposes of this study, tissues from the left filet were subsampled using the snipping procedure. In addition, for fish containing total mercury

exceeding 0.44 mg kg^{-1}, subsamples of homogenized tissue (the right filet) were also analyzed for total mercury concentration.

Analytical quality was monitored by including control samples (reference materials and duplicates) with each batch of test samples. Daily results of these samples were used to confirm the integrity of individual batches of results and to assess the overall performance of the method. In particular, data from duplicate analyses and from the National Research Council of Canada, certified reference material DORM-1 (dried Dogfish Muscle Tissue) were used to assess method precision and accuracy.

The average recovery for total mercury in the certified reference material DORM-1 was 109% with a relative standard deviation (RSD) of 7% (N = 29). The certified value is 0.798 mg kg^{-1} (on the dry weight basis) with a RSD of 9.2%. The average recovery for organic mercury in the certified reference material DORM-1 was 102% with a RSD of 6% (N = 10). The certified organic mercury is 0.731 mg kg^{-1} (on the dry weight basis) with a RSD of 8.2%.

Based on the mean values of the inter-laboratory study conducted by Fisheries and Oceans Canada's Freshwater Institute, Winnipeg, Manitoba, the recovery of total mercury for 12 homogenized wet fish samples was 100-107% with a mean value of 103%. The total mercury level in these samples ranged from 0.1-1.0 mg kg^{-1} on the wet weight basis.

The within-run precision derived from duplicate analysis of homogenized samples was comparable to that of snipping samples in which adjacent snips were taken and analyzed. The between-run precision for aqueous standards or in-house fish reference materials was consistently better than 7% in both total and organic mercury analysis. The estimated overall precision of the total and organic mercury analysis with the snipping sub-sampling procedure was 7.3-11.5%.

3.5 Data Interpretation

In Canada, there is a federal guideline of 0.5 mg kg^{-1} (muscle tissue on the wet weight basis) for commercially marketed fish (Health and Welfare Canada, 1990). This is often taken as a maximum consumption guideline for fish caught by angling. Fish containing between 0.5 and 1.0 mg kg^{-1} may be consumed in limited amounts whereas consumption of fish with more than 1.0 mg kg^{-1} is generally discouraged (MOE, 1991). Because the developing fetus retains more mercury than adults, pregnant women should not consume fish with more than 0.5 mg kg^{-1} mercury. The same restriction generally applies to young children. In the USA, a consumption

guideline of 1.0 mg kg^{-1} is generally applied, whereas guidelines as low as 0.1 mg kg^{-1} have been used in Australia (US EPA, 1989; Western Australia Dept. Conservation and Environment, 1981). In all cases, the guidelines are well below the concentrations (>10 mg kg^{-1}) known to induce symptoms of mercury poisoning following the consumption of tainted fish. Thus administrators and policy makers have some flexibility in developing consumption recommendations.

For the purposes of this report, the guideline of 0.5 mg kg^{-1} will be used to interpret data from 1991. In subsequent years, it may be necessary to use the graduated approach to guideline use, particularly if mercury concentrations in fish significantly increase.

4.0 RESULTS

4.1 Species Inventory

Fish were collected from six sites within the basin: Crowsnest River above Lundbreck Falls (Site I), Oldman River upstream of reservoir (Site II), Oldman Dam reservoir, west basin (Site III), Oldman Dam reservoir, central basin (Site IV), Oldman River immediately below dam (Site V), Oldman River at Fort Macleod (Site VI) (Figure 1). The species and numbers of fish kept for evaluation are listed in Table 1.

4.2 Mercury Concentration in Fish

Of the 656 fish kept, 409 were assayed for total mercury concentration and of these, 123 for organic mercury concentration (Table 2). Regression of organic mercury on total mercury accounted for 97% of the total variation of organic mercury, and approximately 95% of total mercury was estimated to be organic mercury (Figure 2). The organic mercury fraction ranged from 92-104% among species, and ranged from 87-100% among sites.

In general, bull trout, white sucker, and longnose sucker carried larger mercury concentrations than rainbow trout and mountain whitefish (Table 2). Only a small number of fish, caught in the Oldman River immediately below the dam, contained total mercury concentrations exceeding 0.5 mg kg^{-1}. No species or site means exceeded 0.5 mg kg^{-1}.

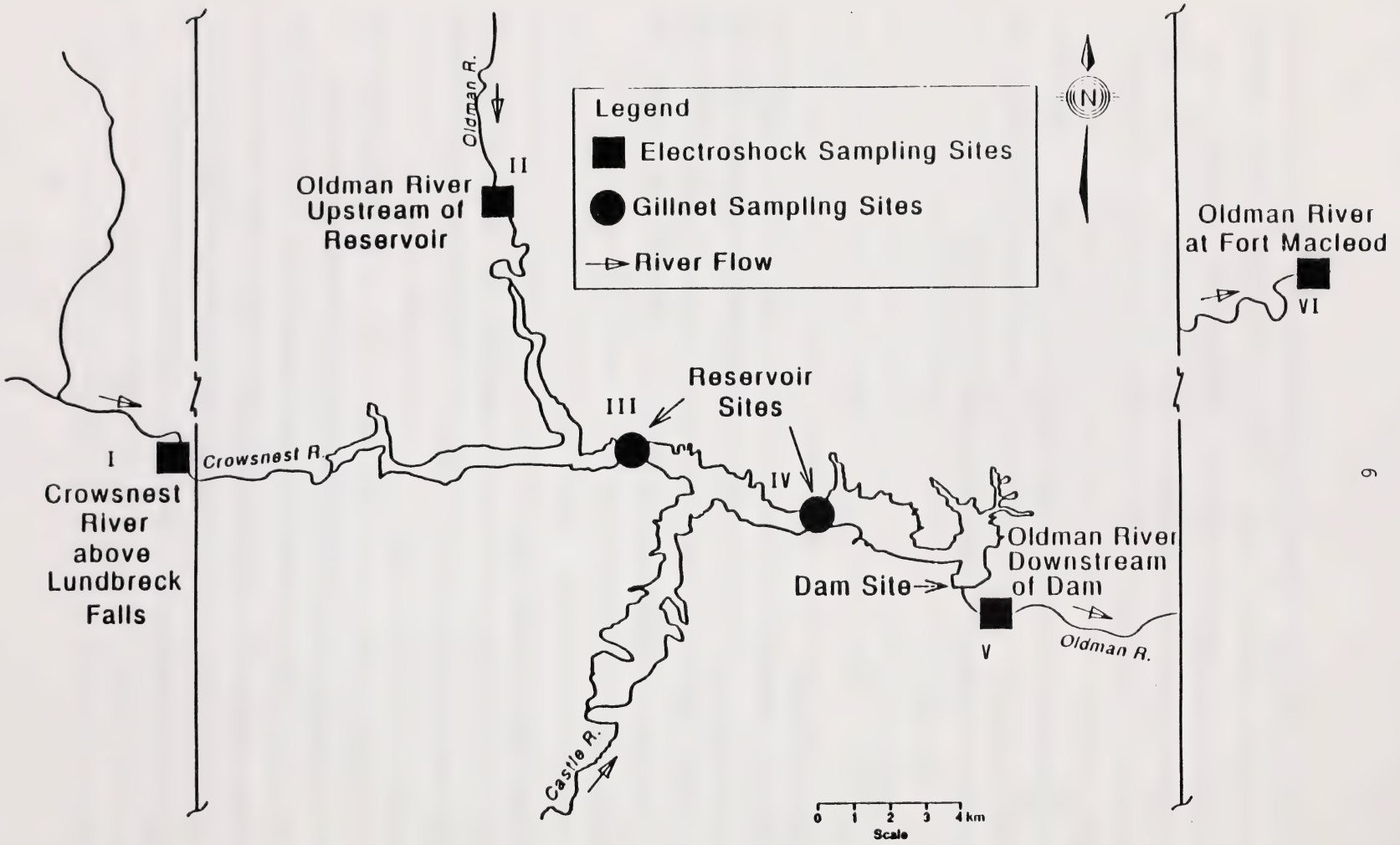

Figure 1. Collection sites I-VI.

Table 1. Species and number of fish kept for evaluation

Site		Species									Total
		Rainbow trout	Mountain whitefish	Bull trout	Brown trout	White sucker	Longnose sucker	Northern pike	Burbot	unidentified cyprinidae	
I	Crowsnest River above Lundbreck Falls	23	20	-	-	-	-	-	-	-	43
II	Oldman River, upstream of reservoir	14	3	3	-	4	4	-	-	-	28
III	Reservoir, west basin	10	100	32	3	22	60	-	-	-	227
IV	Reservoir, central basin	8	47	11	-	16	149	-	-	3	234
V	Oldman River, immediately below dam	20	20	5	-	10	10	-	-	-	65
VI	Oldman River, Fort Macleod	5	20	4	-	12	10	4	4	-	59
Total		80	210	55	3	64	233	4	4	3	656

Table 2. Summary of mercury concentration in fish sampled in 1991 from six sites in the Oldman River Dam Reservoir, the Oldman River near the reservoir and the Crowsnest River

Site	Species*	Year	Length			Total Mercury			Organic Mercury			N of Total Mercury ≥ 0.5 mg kg^{-1}
			N	Fork Length (cm)		N	Concentration (mg kg^{-1})		N	Concentration (mg kg^{-1})		
				Mean	Range		Mean	Range		Mean	Range	
Site I Crowsnest River, above Lundbreck Falls	RNTR	1991	23	31.4	17.9 - 42.3	23	0.052	0.025 - 0.097	10	0.055	0.032 - 0.095	0
	MNWH	1991	20	31.2	29.0 - 34.5	20	0.051	0.009 - 0.081	10	0.051	0.031 - 0.079	0
Site II Oldman River, upstream of reservoir	RNTR	1991	14	22.7	15.0 - 30.4	14	0.046	0.028 - 0.070				0
	MNWH	1991	3	33.2	31.0 - 34.4	3	0.072	0.070 - 0.074				0
	BLTR	1991	3	38.2	24.2 - 46.4	3	0.230	0.162 - 0.283				0
	WHSC	1991	4	25.3	20.2 - 30.0	4	0.115	0.070 - 0.158				0
	LNSC	1991	3	34.1	16.9 - 43.1	3	0.279	0.060 - 0.398				0
Site III Reservoir, west basin	RNTR	1991	10	32.5	16.5 - 56.4	10	0.155	0.021 - 0.367	6	0.082	0.037 - 0.200	0
	MNWH	1991	37	26.8	17.2 - 40.2	37	0.108	0.032 - 0.277	10	0.122	0.029 - 0.291	0
	BLTR	1991	32	29.9	20.8 - 51.5	32	0.181	0.124 - 0.256	7	0.183	0.131 - 0.235	0
	WHSC	1991	22	31.7	11.3 - 47.4	22	0.196	0.082 - 0.404				0
	LNSC	1991	35	31.1	15.4 - 48.8	35	0.203	0.099 - 0.413	4	0.166	0.139 - 0.214	0
Site IV Reservoir, central basin	RNTR	1991	8	34.0	23.7 - 41.9	8	0.271	0.059 - 0.422	4	0.268	0.176 - 0.394	0
	MNWH	1991	6	25.0	19.5 - 44.2	6	0.198	0.131 - 0.389				0
	BLTR	1991	11	29.7	25.2 - 34.0	11	0.205	0.146 - 0.264	3	0.167	0.131 - 0.222	0
	WHSC	1991	16	31.9	16.1 - 45.9	16	0.169	0.094 - 0.435				0
	LNSC	1991	35	23.7	15.7 - 38.0	35	0.200	0.110 - 0.451	15	0.175	0.096 - 0.263	0
Site V Oldman River, immediately below dam	RNTR	1991	20	30.6	23.4 - 39.1	20	0.325	0.132 - 0.559	11	0.311	0.142 - 0.458	1 (3)‡
	MNWH	1991	20	27.3	18.5 - 36.7	20	0.157	0.083 - 0.267	9	0.154	0.096 - 0.228	0
	BLTR	1991	5	35.2	30.0 - 42.3	5	0.281	0.218 - 0.348	5	0.263	0.233 - 0.310	0
	WHSC	1991	10	37.6	34.6 - 41.3	10	0.376	0.241 - 0.522				2 (2)‡
	LNSC	1991	10	41.4	28.7 - 48.6	10	0.280	0.127 - 0.413				0
Site VI Oldman River, Fort Mcleod	RNTR	1991	5	33.7	30.1 - 37.7	5	0.163	0.074 - 0.225	5	0.161	0.083 - 0.224	0
	MNWH	1991	20	28.8	20.8 - 38.0	20	0.125	0.083 - 0.271	10	0.133	0.085 - 0.280	0
	BLTR	1991	4	32.5	29.2 - 37.0	4	0.209	0.171 - 0.241	4	0.196	0.160 - 0.232	0
	WHSC	1991	12	35.9	26.0 - 42.8	12	0.236	0.054 - 0.479				0
	LNSC	1991	10	40.9	37.5 - 43.0	10	0.297	0.219 - 0.443	10	0.305	0.248 - 0.460	0
	NRPK	1991	4	27.8	22.6 - 36.9	4	0.231	0.189 - 0.286				0
	BURB	1991	4	59.8	53.0 - 64.6	4	0.291	0.121 - 0.427				0

[§]Analysis performed on sub-samples by snipping procedure.

* RNTR - Rainbow Trout MNWH - Mountain Whitefish BLTR - Bull Trout WHSC - White Sucker
LNSC - Longnose Sucker BNTR - Brown Trout NRPK - Northern Pike BURB - Burbot

‡ denotes number of fish ≥ 0.44 mg kg^{-1} (snipped) and also ≥ 0.5 mg kg^{-1} (homogenized).

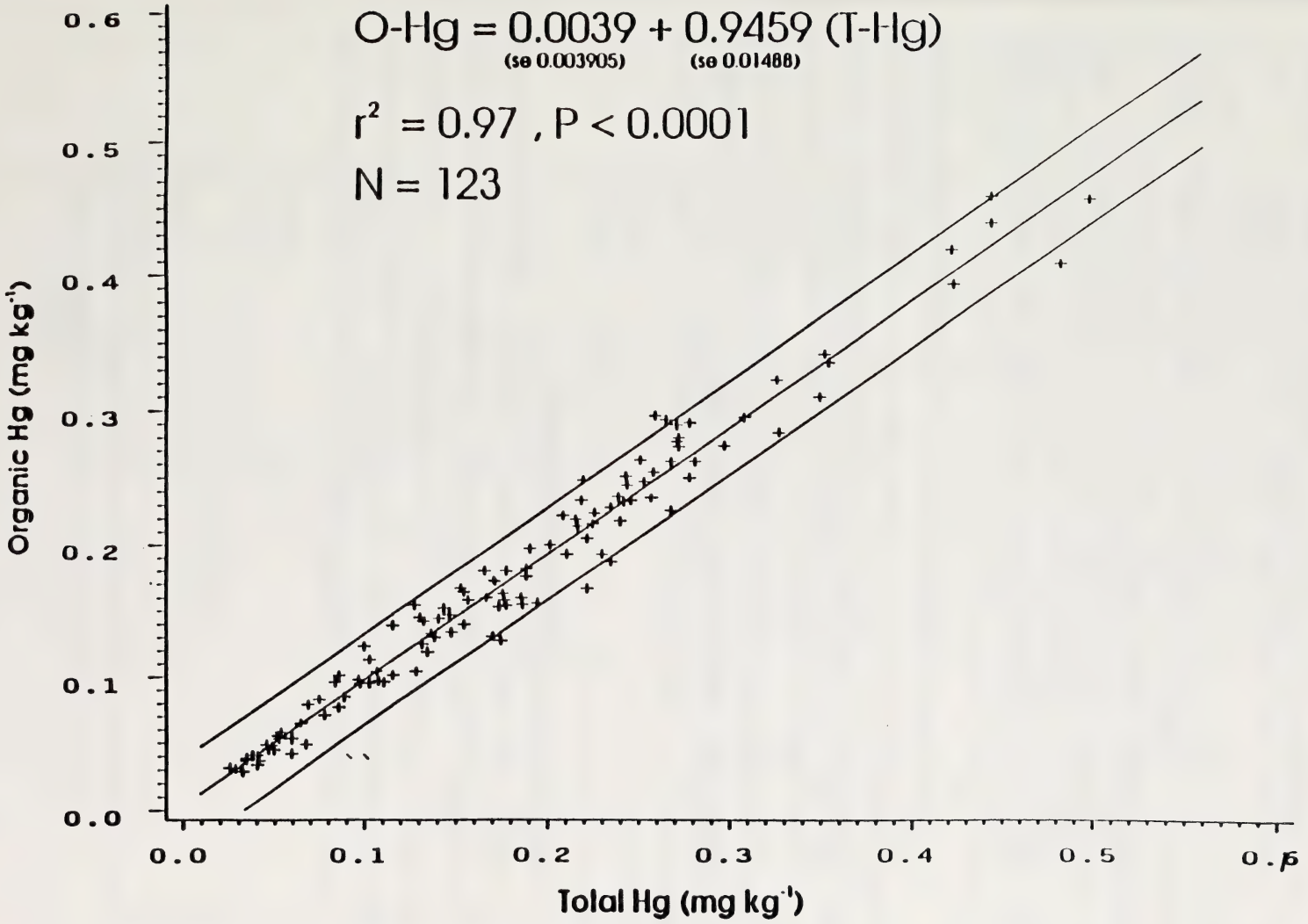

Figure 2. Regression of organic mercury on total mercury for fish caught in 1991.

Total mercury assayed in all species varied significantly among sites but not between sexes whereas Site * Sex interactions were significant only for longnose sucker, mountain whitefish and rainbow trout (Table 3). Because average size differences due to sex (males and females, only) were not statistically different (Table 4), pooled data were used in correlation analyses of size and total mercury (Table 5). Mercury concentrations increased with size for rainbow trout, white sucker and longnose sucker, but, longnose sucker collected at site IV (central reservoir), had a negative relationship between total mercury concentration and size (Table 4). Just below the dam (site V) this species exhibited a positive trend between mercury concentration and size.

After adjusting means of total Hg for variation in sample sizes and fish sizes, significant Site*Species interactions were detected at $P \leq 0.0001$, indicating average mercury content for each species to be dependent on sampling site. In general, mercury content of some species increased from west to east, maximized immediately below the dam in the Oldman River, and decreased at the eastern most site near Fort Macleod (Figures 3-7). Good examples of the Site*Species interaction can be seen where we compare the site means for rainbow trout and mountain whitefish (Figures 3, 4, respectively) versus those of bull trout (Figure 5): note the shift pattern at sites III and V.

4.3 Fish Size, Growth, Feeding Habits, and Fecundity

Average caught weight (C-WT) and length (C-LEN) did not differ by site or sex for bull trout and white sucker (Table 4). Both size parameters differed equally for the sexes among sites for rainbow trout and mountain whitefish, while significant interactions between site and sex for longnose sucker indicate that size differences between the sexes changed dependent on sampling site. Analysis on age, growth and feeding habits for 1991 samples have not been completed yet.

Table 3. Summary of analysis of variance model of total mercury concentration (mg kg^{-1}) among five fish species sampled from six sites and by sex, including immatures (sex unknown)[§]

Source	Rainbow trout	Mountain whitefish	Bull trout	White sucker	Longnose sucker
Site	***	***	***	***	*
Sex	ns	ns	ns	ns	ns
Site * Sex	*	**	ns	ns	***

[§] Level of statistical significance: *(0.05); **(0.01); ***(0.001)
ns: not significant

Table 4. Size and weight analyses by species among sites; data for Y= log C-WT or log C-LEN were entered into the following factorial model: Y = Site + Sex + Site*Sex[§]

	Sites	Sex	Site*Sex
	Rainbow trout		
Weight	***	ns	ns
Length	***	ns	ns
	Mountain whitefish		
Weight	***	ns	ns
Length	***	ns	ns
	Bull trout		
Weight	ns	ns	ns
Length	ns	ns	ns
	White sucker		
Weight	ns	ns	ns
Length	ns	ns	ns
	Longnose sucker		
Weight	***	ns	**
Length	***	ns	*

[§] Level of statistical significance: *(0.05); **(0.01); ***(0.001)
ns: not significant

Table 5. Spearman correlation coefficients for total mercury concentration (mg kg^{-1}) and fish size (caught weight, kg, caught length, cm)

Site	Species	n	Size Parameter	
			Caught Weight	Caught Length
I	Mountain whitefish	20	ns	0.48*
	Rainbow trout	23	0.50**	0.55**
II	Bull trout	3	ns	ns
	Longnose sucker	3	1.00+	1.00+
	Mountain whitefish	3	ns	1.00+
	Rainbow trout	14	ns	ns
	White sucker	4	ns	ns
III	Bull trout	32	ns	ns
	Brown trout	3	-1.00+	-1.00+
	Longnose sucker	36	ns	ns
	Mountain whitefish	36	ns	ns
	Rainbow trout	10	ns	ns
	White sucker	22	0.63**	0.62**
IV	Bull trout	11	ns	ns
	Longnose sucker	35	-0.72**	-0.72**
	Mountain whitefish	6	ns	ns
	Rainbow trout		ns	-0.71*
	White sucker	16	ns	ns
V	Bull trout	5	ns	ns
	Longnose sucker	10	0.88**	0.71*
	Mountain whitefish	21	ns	ns
	Rainbow trout	19	ns	ns
	White sucker	10	0.89**	0.83**
VI	Bull trout	4	ns	ns
	Longnose sucker	10	ns	ns
	Mountain whitefish	20	ns	ns
	Northern pike	4	ns	ns
	Rainbow trout	5	ns	ns
	White sucker	12	0.72**	0.64*

* Significantly different from zero at 0.05 level
** Significantly different from zero at 0.01 level
\+ Caution: Extremely small sample size
ns: not significant

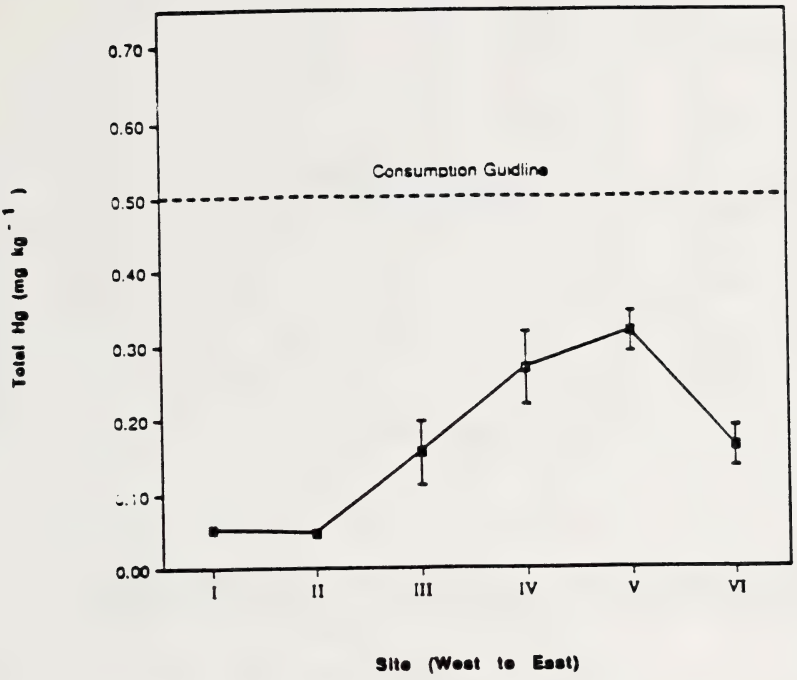

Figure 3. Rainbow trout. Mean (±s.e.) of total mercury among sites

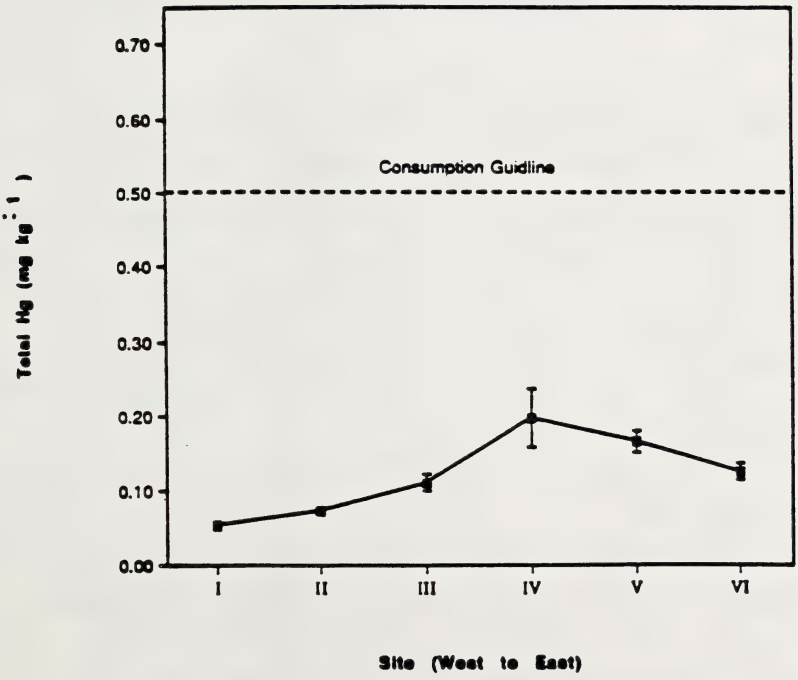

Figure 4. Mountain whitefish. Mean (±s.e.) of total mercury among sites

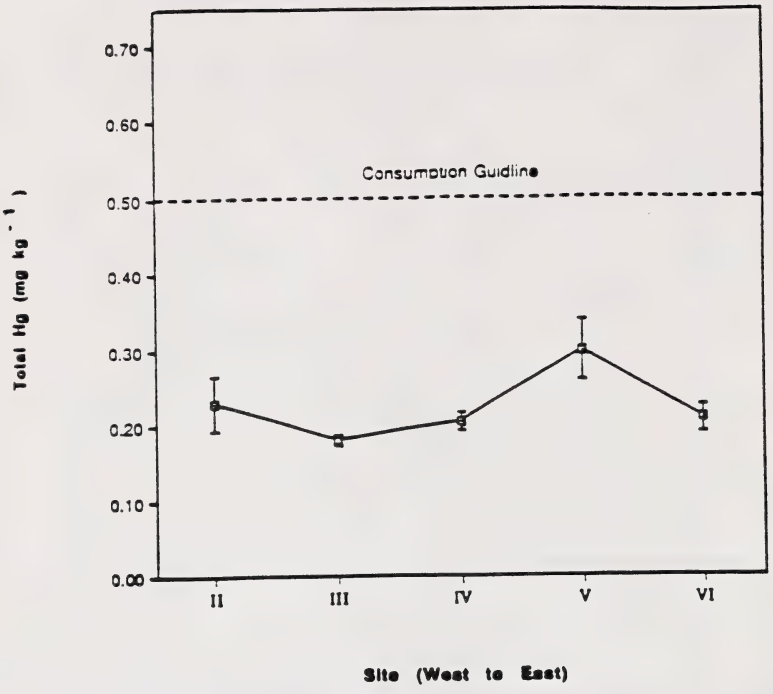

Figure 5. Bull trout. Mean (±s.e.) of total mercury among sites

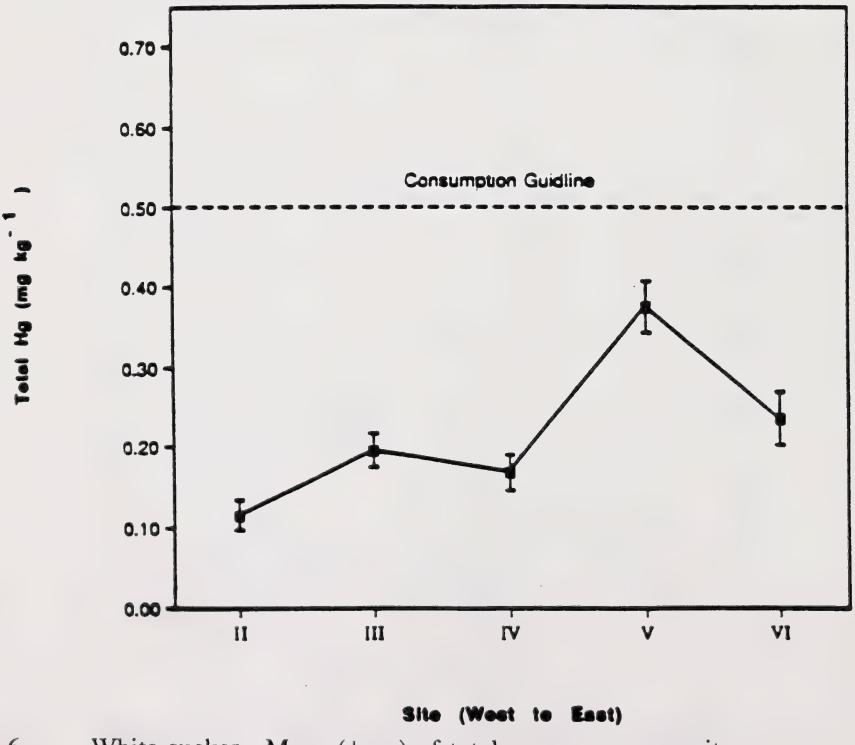

Figure 6. White sucker. Mean (±s.e.) of total mercury among sites

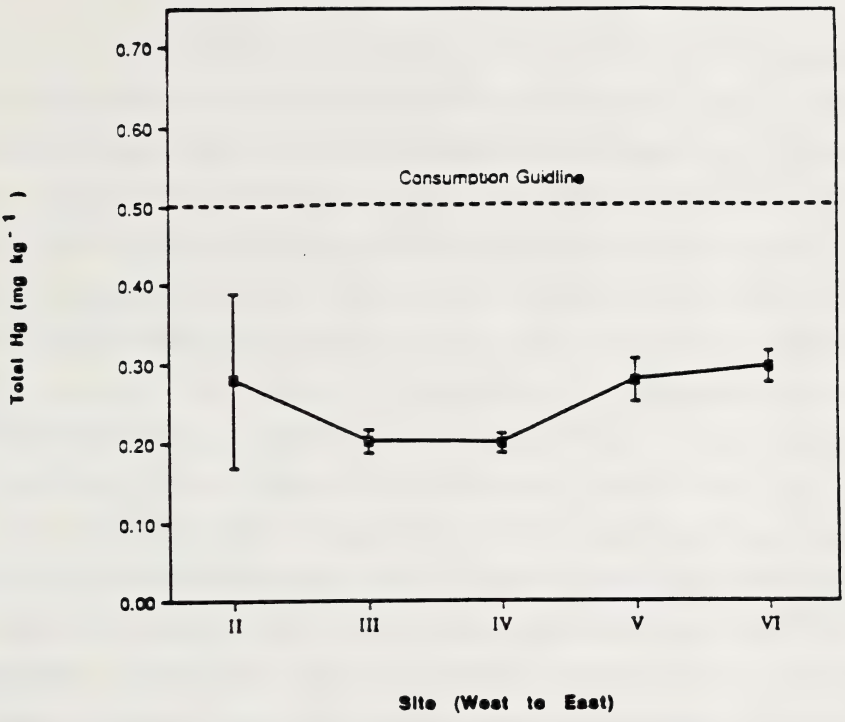

Figure 7. Longnose sucker. Mean (±s.e.) of total mercury among sites

Table 6. Summary of ranges of total mercury concentration (mg kg^{-1}) and sample size (N) from five fish species sampled upstream of the Oldman Dam site and downstream of the dam site, April 1986 (Alberta Environment, 1989)

LOCATION	Rainbow trout		Mountain whitefish		Bull trout		White sucker		Longnose sucker	
	N	Range	N	Range	N	Range	N	Range	N	Range
Upstream of the dam	23	0.076 - 0.305	30	0.077 - 0.406	5	0.177 - 0.304	9	0.142 - 0.518	26	0.241 - 0.528
Downstream of the dam	7	0.092 - 0.285	10	0.072 - 0.312	na	na	10	0.320 - 0.750	10	0.113 - 0.442
Overall	30	0.076 - 0.305	40	0.072 - 0.406	5	0.177 - 0.304	19	0.142 - 0.750	36	0.113 - 0.528

na: not available for analysis

5.0 DISCUSSION

Since only one year of data has been collected, care must be taken in interpreting the above-noted findings. Limnological conditions in the reservoir and Oldman River downstream of the reservoir are currently dynamic and will not stabilize for several years. As changes occur in physical and chemical conditions (pH, redox potential, concentration of sulphides, manganese and iron hydroxides, and trophy) of the sediments and water column, the availability of organic mercury to aquatic organisms may also change. Similarly, the resident fish populations, formerly riverine, must adapt to changing reservoir conditions or move upstream to one of three rivers (Oldman, Crowsnest, Castle). Different populations of fish species probably inhabited the reservoir during 1991, and we are currently unable to segregate our catch according to the original site of the fish. The inventory, growth and mercury data may have to be interpreted according to origin of the populations of different species, especially as the study progresses.

Bull trout, a rare species throughout most of Alberta, appeared to be abundant in the reservoir during 1991. In most cases, bull trout prefer lotic conditions, so we might expect gradual depopulation of this species over the next several years. Also, mountain whitefish may migrate upstream, as was noted in the Dickson Dam reservoir located on the Red Deer River upstream of the City of Red Deer (Alberta Environmental Centre, 1989b). A similar study on Southern Indian Lake, Manitoba (Bodaly *et al.*, 1984b) also attributed the post-impoundment collapse of the lake whitefish (*Coregonus clupeaformis*) to emigration from the reservoir. The other major game species in the Oldman River Dam reservoir, including rainbow trout, may populate the reservoir, particularly if suitable spawning sites and habitat are available.

Total mercury concentrations in fish were relatively low during 1991, and posed no threat to human consumers. This was anticipated considering the early stage of development of the reservoir and the species complex of fish, dominated by trout, whitefish and suckers which are generally omnivorous or secondary carnivores. Although we cannot predict if residues will increase as the reservoir ages as discussed above, no such increase was noted over five years in Gleniffer Lake at the Dickson Dam (Alberta Environmental Centre, 1989a). Gleniffer Lake is geographically closer to the Oldman River system than any other newly formed reservoir in Alberta, and seems to be the best model available for comparison.

Bull trout, containing among the highest mercury concentrations of any species in the Oldman River system. is probably at the top of the food chain. This species is often piscivorous and could consume any other fish in the reservoir, thereby enhancing biomagnification of mercury. The suckers, on the other hand, are in the middle of the food chain, feeding on benthic invertebrates and soft substrates. The relatively high mercury concentrations in these species, consistent with pre-impoundment studies (Table 6) may be due to ingestion of organic mercury bound to iron and manganese hydroxides and clay particles. The relatively low residues found in rainbow trout and mountain whitefish are consistent with those reported for fish collected from the lower reaches of the Crowsnest River in 1982 and 1983, and the Oldman River in 1986 well before any reservoir development activities (Alberta Environmental Centre, 1984; Table 6).

The 1991 trend of increasing mercury residues in some species moving from west to east in the Oldman River basin may be due to a number of factors or combination of factors of both natural and anthropogenic origins: (i) increased methylation as the river becomes more eutrophic moving downstream, (ii) increased methylation as the river warms moving downstream, (iii) changes in the physical and chemical properties of sediments, resulting in increased mobilization of inorganic and organic mercury, (iv) change in the diet of fish, (v) sampling artifacts. Without a larger database, it is not possible to interpret this trend at the present time. This data base will be established during the next four years.

Methyl mercury comprises the bulk of mercury in fish tissue (Westöö, 1967). Various studies place the proportion of methyl mercury to inorganic mercury at 80% (WHO, 1990), 90% (Huckabee *et al.*, 1979; May *et al.*, 1987; Hildebrand *et al.*, 1980; Mikac *et al.*, 1985; Jackson, 1991) or >95% (Surma-Aho *et al.*, 1986; Grieb *et al.*, 1990; Bloom, 1992). This range of methyl mercury applies to polluted and nonpolluted environments. Differences in the methyl mercury fraction reported among studies may be real or the result of analytical and sampling artifacts. Some of the environmental factors causing real variability include differences in (i) the rate of methylation of mercury in water and sediments, (ii) the rate of *in vivo* methylation, (iii) concentration metallothionein and other metal-binding proteins in tissues, and (iv) species-dependent differences in the uptake and depuration rate of methyl mercury. Some of the key artificial factors include (i) different analytical methods, and (ii) inadequate or unrepresentative sampling of fish populations. Uniformity in analytical and sampling procedures may assist in resolving this issue.

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Appendix A

Protocol 2440-DL2/P1

(Oldman Dam: Mercury in Fish) [1991]

EXPERIMENTAL PROTOCOL

OLDMAN DAM: MERCURY IN FISH

BY

J.W. MOORE, L.Z. FLORENCE AND B.C. GOSKI

AQUATIC BIOLOGY BRANCH

ANIMAL SCIENCES DIVISION

ALBERTA ENVIRONMENTAL CENTRE

VEGREVILLE, ALBERTA T0B 4L0

September 11, 1991

2440-DL2/P1

OLDMAN DAM: MERCURY IN FISH

NOTICE

Page 1 of the original copy of Experimental Protocol, "Oldman Dam: Mercury in Fish" contains the signatures authorizing approval of the protocol. These signatures included the following:

1. Principal Investigator
2. Branch Head, Aquatic Biology Branch
3. Statistician
4. Head, Animal Resources Section
5. Director, Animal Sciences Division
6. Acting Chairman, Animal Care and Use Committee

I. ADMINISTRATIVE INFORMATION.

A. Program: Ecological Studies (DL)

B. Project/Sub-Project: Aquatic Habitat Studies (DL2)

C. Study Title, AEC Number, Author(s) and Starting and Ending Dates.

1. Title: Oldman Dam: Mercury in Fish
2. Number: 2440-DL2/P1
3. Authors: J.W. Moore, L.Z. Florence and B.C. Goski
4. Word Processing File I.D. 1914G
5. Date Written: March 8, 1991
   Date Revised: March 28, 1991
6. Date of ACUC Approval:
7. Starting Date.
   a. Anticipated: September/October, 1991
   b. Actual:
8. Ending Date.
   a. Anticipated: March, 1997
   b. Actual:
9. Duration: 6 years

D. Client Department, Contact Person and Date for Final Report.

1. Client Department(s): Alberta Public Works, Supply and Services; Reservoir Development Division and Environmental Quality Monitoring Branch of Alberta Environment; Fish and Wildlife Division, Forestry, Lands and Wildlife
2. Contact Person: J.W. Thiesen; B. Kemper; M. Drouin
3. Date Final Report Due: December, 1997

E. Principal Investigator(s), Participants and Levels of Responsibility. (Note: Original copy of experimental protocol contains names of Principal Investigator and Participants)

1. Principal Investigators:

2. Participants:

3. Responsibility:
   a. Statistics:
   c. Q/A, Q/C:
   d. Mercury Analysis: AEC Chemistry/Private Laboratory
   e. Writing Report(s):

F. Location of Study: Fish collections at the Oldman River Dam Reservoir and Oldman River near Pincher Creek, Alberta.

Sample analysis at the Alberta Environmental Centre or private laboratory.

G. Test Agent and Hazard: No test agent

H. Safety:

The Oldman reservoir will be exposed to high wind velocities, creating boating hazards. Appropriate boats and water safety equipment for fish collections are necessary. All staff involved in field collections will require certified training courses in boat handling and water safety. Aquatic Biology Branch staff will require refresher courses.

I. Animals and Husbandry.

1. Animals.
   a. Species: The following species may occur in the reservoir. Rainbow trout Oncorhynchus mykiss, cutthroat trout Salmo clarki, northern pike Esox lucius, mountain whitefish Prosopium williamsoni, longnose sucker Catostomus catostomus, white sucker Catostomus commersoni, mountain sucker Catostomus platyrhnchos, burbot Lota lota and dolly varden Salvelinus malma. At present it is not known which species will be captured in the nets. If a species is captured, it will be selected for study.
   b. Strain or breed: Not known
   c. Sex: To be determined after collection
   d. Body weight at start of test: To be determined after collection
   e. Length: To be determined after collection
   f. Age: To be determined after collection using scales, fin rays, opercula, and otoliths
   g. Acclimation/Acclimatization: Not applicable
   h. Number of animals: Yearly maximum of 2592 fish
   i. Disposal of fish: SOP No. 2350-AJ4/PR/NEC/6/91

2. Husbandry.
   a. Housing and Caging: Not applicable
   b. Feed: Not applicable
   c. Water: Not applicable
   d. Animal care SOPs: Not applicable
   e. Animal identification: Each fish identified by Animal Sciences Accession Number and Aquatic Biology Branch Number

II. Background, Objectives and Experimental Design.

A. Background.

The construction of large dams often results in major ecological changes within river basins. In some cases, the growth of fish in the reservoir may increase, resulting in the development of strong commercial, domestic and sport fisheries. Some fish species may wax in abundance whereas others may wane to negligible proportions. Fisheries managers often take advantage of these changes by introducing desirable species such as salmonids and percids.

One potential drawback of reservoir construction is the possible increase in mercury levels in fish inhabiting the reservoir.

The adulteration of fish tissues with mercury first became a pervasive environmental issue approximately 30 years ago with the poisoning of people around Minamata Bay, Japan (Mitra, 1986). The symptoms of the poisonings were striking, affecting the central nervous system, and producing birth defects and death in severe cases. Although the magnitude of Minamata disease (as it is now termed) stimulated agencies around the world to regulate mercury discharges, several other less severe cases of poisoning were reported among fishermen and others in several countries during the 1960s and early 1970s (Mitra, 1986). This further stimulated regulatory agencies to control and monitor mercury contamination in the environment.

In recent years, most western nations have developed regulations which either restrict or severely limit the quantity of mercury discharged from point sources. Although this has greatly reduced the level of environmental contamination of mercury in most areas, nonpoint sources of mercury continue to be difficult to regulate. Geologic formations may be naturally high in mercury, particularly in areas of volcanic activity (Jonasson and Boyle, 1972). The western part of Alberta lies in one such belt.

Newly formed reservoirs are a nonpoint source of mercury. Immediately after flooding, the rate of methylation in freshly inundated soils increases which, in turn, increases uptake of organic mercury in fish and other aquatic species (Cox et al., 1979). This increase in mercury has been noted in many impoundments in Canada and several other places in the world (Benson et al., 1976; Bodaly et al., 1984; Bodaly and Hecky, 1979; Bruce and Spencer, 1979; Cox et al., 1979; Kent

and Johnson, 1979; Knight and Herring, 1972; Meister et al., 1979; Potter et al., 1975; Smith et al., 1974).

Residues of over 3 mg kg^{-1} in fish tissues have been reported in some of the above-noted studies. This can be compared to the consumption guideline in Canada of 0.5 mg kg^{-1} (Ontario Ministry of Environment, 1983). Eventually reducing conditions develop in older reservoirs, causing a decrease in the activity of methylating bacteria and the amount of organic mercury available to fish and other aquatic species (Cox et al., 1979).

B. Objectives.

The objectives of this study are to:

1. document changes in the species complex of fish inhabiting the reservoir,

2. determine changes in growth, size and abundance of fish in the reservoir,

3. assess changes in the mercury content of fish muscle tissue over a five year period in the reservoir and river.

C. Experimental Design.

Experimental Design and Sampling

1. The following linear statistical model, and sample sizes, are based upon previous surveys in the Oldman River basin; the most likely sources of variation in a stratified sampling scheme are:

$$y = \mu + \text{Location} + \text{Site} + \text{Depth} + \text{Location} * \text{Site} + \text{Location} * \text{Depth} + \text{Site} * \text{Depth} + \text{Location} * \text{Site} * \text{Depth} + \text{Error (random)}$$

where, y is the species count or proportion or Hg content of a fish, μ is the overall, true population mean and Location, Site and Depth represent spatial strata of sampling. After one year's sampling (fall, 1991), subsequent models will include "year" as an additional source of variation.

2. Sample Sizes

Five locations above and 3 locations below (noted in Fig.1) the dam will each contain two sites (each within 500-1000 m of the other), and where possible, two depths at each site

(Table 1) (Figure 1). For each species, at least 9 animals per depth will be sampled; thus, at each location, 36 animals (2•2•9) will be sampled (all animals of a species will be counted to estimate variation in proportions among species and locations).

Source	No. of Levels	Degrees of Freedom	Power*
Location (L)	8	7	1.0
Site (S)	2	1	1.0
Depth (D)	2	1	1.0
L * S	-	7	0.85
L * D	-	7	0.85
S * D	-	1	0.98
L * S * D	-	7	0.85
Error	288	255	-

*Expectations based on effect sizes determined from previous studies on the Oldman River basin. See Cohen (1988) for methods and discussion.

Therefore, if present, 288 animals total, per species, may be sampled over 8 locations. While these are quite large expectations, these numbers would provide a probability of detecting a significant result (power of the test), for each term in the model, of 0.85, or greater, while keeping the Type I error (significance level) at $\leq$0.05. This would also permit detecting a positive correlation between Hg content and fish size of $\geq$0.204 (given the true correlation is $\geq$0.30) with probability of $\geq$0.80, and one-tailed Type I error rate of $\leq$0.05.

3. While alternative sampling schemes can be designed, having minimum acceptable power of detection, considerable precision may be lost and likely sources of variation must be neglected by pooling over depths, sites and/or locations. Alternative methods should only be considered after the first year of sampling.

4. Because depth in rivers, above and below the reservoir, may not permit a "shallow" and "deep" setting of nets, comparisons should be made, at one or more locations, between netting and electroshocking fish. This could assist us in correcting spurious estimates due to the anticipated paucity of fish in the reservoir.

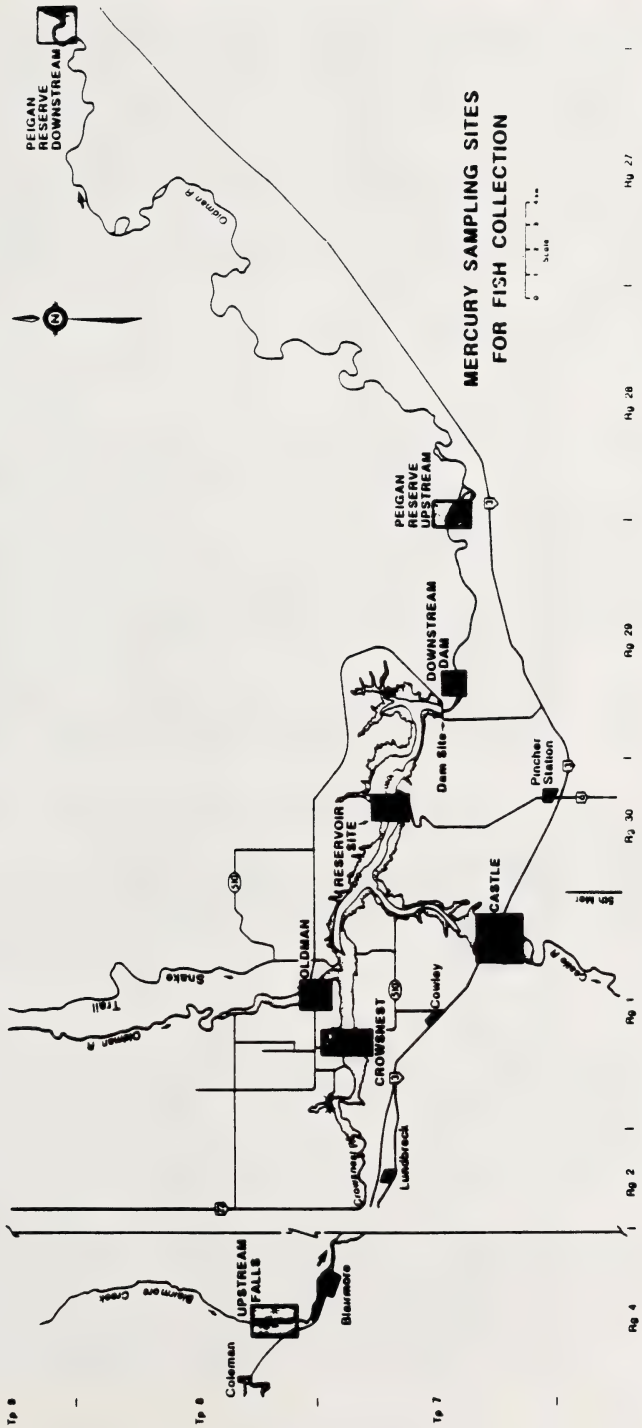
MERCURY SAMPLING SITES
FOR FISH COLLECTION
PEIGAN RESERVE DOWNSTREAM
PEIGAN RESERVE UPSTREAM
DOWNSTREAM DAM
Dam Site
Pincher Station
RESERVOIR SITE
CASTLE
OLDMAN
CROWSNEST
Cowley
Lundbreck
UPSTREAM FALLS
Blairmore
Coleman
Oldman R.
Crowsnest R.
Snake Trail
Ranges
Townships

Figure 1.

5. Given the level of sampling intensity planned in 1991, it will be possible to monitor changes in the frequency of a species above the dam vs. below the dam, with 80% probability of detecting a true change, assuming for example:

   a. if a species is rare or very frequent (0.10 or 0.90), we can detect changes of 10-15 percent, $\alpha \leq 0.05$;
   b. if a species is at intermediate (40-50%) levels in one population, we can detect shifts of 15-20%, with $\alpha \leq 0.05$.

6. Fish Collection (Reservoir):

   a. Fish will be collected using multi-panel gill nets, measuring 50 m in length, 1.8 m in depth and made of green or colourless monofilament nylon. Mesh sizes will be 2.5, 3.8, 5.1, 6.4, 7.6, 8.9, 10.2, 11.4 and 12.7 cm. All mesh sizes will be used. Two nets will be fished together yielding a total net length of 100 m.
   b. Electroshocking, seine netting and angling may be necessary to supplement small catches of certain species.
   c. Size of fish that will be collected is unknown.
   d. Live fish will be euthanized as per SOP 2350-AJ4/PR/Euth/1.
   e. Collections will be made yearly during September and October.
   f. Nets will be set and left for 24 h, weather permitting.
   g. Healthy fish, excessive to the needs of the study, will be released.

7. Fish Collection (Oldman River):

   a. Depending on water flow, fish will be captured using gill nets, seine nets, angling or electroshocking.
   b. Gill nets will be set and left for 24 h, weather permitting.
   c. All live fish will be euthanized as per SOP 2350-AJ4/PR/EUTH/1.
   d. Collections will be made yearly during September and October.
   e. Healthy fish, excessive to the needs of the study, will be released.

8. Tissue Preparation:

   a. The following data will be obtained from each fish collected (Moore, 1990):
      i) Identified to species
      ii) Sexed
      iii) Sexual maturity
      iv) Weighed

v) Fork length
vi) Aged (scale, fin ray, opercula, otolith)
vii) Stomach contents
viii) ovaries.

b. A 30 - 100 g sample of boneless, skinless dorsal muscle fillet will be dissected for mercury analysis from an area anterior to the dorsal fin of each fish within 6 h of collection.

c. Samples will be wrapped in distilled, water-rinsed aluminum foil, placed in individual plastic bags and labelled appropriately.

d. Tissues will be frozen to -20° C pending analysis.

9. Mercury Analysis

a. Mercury analysis will be completed on fish from both the reservoir and river. The number of analyses will depend on the level of funding for this aspect of the work, species present, the number of fish collected, and the size of fish collected. The number of fish used in mercury analysis will be equal to or less than the number used for the growth and inventory aspects of this work. The level of funding for this aspect of the study is unknown at this time.

b. The protocol for the mercury analysis of fish will be prepared by Chemistry Division.

10. Physico-Chemical Analysis

a. The pH, dissolved oxygen (DO), temperature, conductivity of the water will be determined at each collection site immediately after the nets are raised. Determinations will be made at 5-m intervals.

11. Data Interpretation

a. Weight, length, age, feeding and fecundity data will be used by Alberta Environmental Centre staff (AEC) to determine growth and productivity of fish species in the reservoir, which, in turn, will be used by the clients to assess the need for fish plantings and establishment of commercial/domestic fisheries.

b. CPUE (catch per unit effort) will be used by AEC staff to determine relative changes in fish abundance over the course of the study. This information will also be used by the clients to assess the feasibility of fish plantings and commercial/domestic fisheries.

c. Mercury residue data will be used by the clients to regulate human consumption of fish.

III. References.

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ALBERTA ENVIRONMENTAL CENTRE ANIMAL UTILIZATION FORM

Date 28 March 1991 Protocol No. 2440-DL2/P1 Principal Investigator B. Goski/J. Moore

Species Multiple fish species Date Statistician Approval

Number of Animals Maximum 2592/year Housing N/A Identification N/A

Source Oldman Reservoir and River Caging N/A Average Weight N/A

Wildlife Permit No. Bedding N/A Age (approximately) N/A

Sex Unknown Food Unknown Other (specify) N/A

Arrival Date N/A Water N/A

Date Experimental Procedures to be Started September 1991

Test Substance None Anesthesia/Analgesia MS222

Describe Experimental Procedures and Criteria for Ending Study (see back side): Capture of fish from the Oldman Dam and River. Analysis of growth, relative abundance and mercury in tissues.

Describe Method(s) of Euthanasia: MS222

SIGNATURES

Animal Care Committee	Date	Principal Investigator	Date	Branch Head	Date

Determination of growth, relative abundance and mercury in fish from the Oldman Dam and River.

Description of Invasive or Manipulative Procedures

Muscle tissue will be removed from fish anesthetized with MS222.

Signature of Person Doing These Procedures _____ Date _____

Animals Used or Housed Away From AEC

Describe Animal Care: N/A

Signature of Person Responsible for Animal Care N/A _____ Date _____

Other

Signature of Person Responsible _____ Date _____

RWC/vjg/PARA.AS.UTIL.SHEET/424-C4/87.03.26

ADDENDUM TO PROTOCOL 2440-DL2/P1

Annual Budget - Oldman Dam: Mercury in Fish
(Aquatic Biology Component)

NEW BUDGETARY ITEMS

	Thousands
Operating	
Subsistence	9.6
Travel	1.0
Gill nets	1.5
	12.1
Salaries	
Wage positions	
-new positions (2-6 months)	25.0
Fixed Assets	
Lap top computer (first year only)	3.5
	40.6

EXISTING ITEMS

Fixed Assets	
Boston Whaler	20.0
River Boat	15.0
Water quality instrumentation	8.0
Sampling Equipment	5.0
Vehicles (2)	-
Salaries of Existing Staff	
Professional (0.5 FTE)	30.0
Technical (0.65 FTE)	26.0

JWM/grf
1914G
91.07.09

Appendix B

Amendments to and deviations from Protocol 2440-DL2/P1

Alberta
ENVIRONMENTAL CENTRE

MEMORANDUM

BAG 4000, VEGREVILLE, ALBERTA T9C 1T4

FROM J.W. Moore
Branch Head
Aquatic Ecology

OUR FILE REFERENCE 2440-DL2

YOUR FILE REFERENCE

TO File DL2
(Oldman Dam: Mercury in Fish)

DATE September 28, 1992

TELEPHONE Ext. 8267

SUBJECT Amendments to and Deviations from Protocol 2440-DL2/P1 (Oldman Dam: Mercury in Fish)

During implementation of the above-noted protocol during 1991, the following amendments were made:

i) Page 5, Item C. Experimental Design; 2. Sample Sizes.

Only two sample sites above the reservoir (Crowsnest River above Lundbreck Falls, Oldman River upstream of reservoir) and two sample sites below the reservoir (Oldman River immediately below dam, Oldman River at Fort Macleod) were sampled outside of the reservoir. Only two sites were sampled in the reservoir (reservoir-west basin, reservoir-central basin).

ii) Page 8, Item C. Experimental Design; 6. Fish Collection (Reservoir)

Only gill nets were used to catch fish.

iii) Page 8, Item C. Experimental Design; 7. Fish Collection (Oldman River)

Fish were collected from the Oldman River and Crowsnest River as noted preceding. Only electroshocking was used to collect fish.

iv) Page 8, Item C. Experimental Design; 8. Tissue Preparation

Fish were not wrapped in aluminum foil; fish were wrapped in plastic bags prior to freezing.

J.W. Moore

JWM/lmy
M6DLY140.MEM

Appendix C

Analytical Method: Total Mercury in Fish Tissue

TOTAL MERCURY IN FISH TISSUE

(Acid Digestion, Cold-Vapour Atomic Absorption Method)

Introduction

Mercury can be released into aquatic systems from natural and anthropogenic sources. Regardless of the chemical species introduced, mercury is subject to methylation by microbial and chemical activity. Methyl mercury is readily taken up by fish either directly from the water or indirectly through food. Since the elimination rate of methyl mercury from fish is known to be slow, mercury can rapidly accumulate to relatively high levels.

Consumption of fish high in mercury is of particular concern in that methyl mercury is exceptionally toxic to man and is the predominant mercury species found in the edible portion of fish. In Canada, the Health Protection Branch of Health and Welfare Canada recommends a limit of 0.5 mg/kg total mercury in the edible portion of fish offered for sale.

Summary of Method

Total mercury is determined by gradually digesting fish tissue to 250°C in a mixture of concentrated sulphuric and nitric acids. The digestion converts organic and inorganic mercury to mercury(II) which in turn is reduced to elemental mercury with tin(II). Evolved mercury vapour is then swept with nitrogen through a UV mercury monitor where absorbance is measured at 253.7 nm. The absorbance is compared to the absorbances of a series of mercury standards and converted to mercury concentration in fish tissue.

Scope

This method is applicable to the analysis of mercury in fish muscle tissue and may be applicable to whole fish, fish organs, or to other animal tissues. Based on a practical sample size of 0.4 g, the analytical range is 0.02 - 1.25 mg/kg mercury on a wet weight basis. The range can be extended by dilution of the sample digest or by reducing the weight of the sample.

Interferences

1. Water vapour condensing on the lenses of the mercury monitor can cause interfering noise and signal drift. Warming the absorbance cell to 50 - 60°C limits this to a degree. The cell and connecting gas lines should be inspected and cleaned regularly to prevent substantial moisture accumulation.
2. Organic vapours (such as acetone or benzene) will cause interference by absorbing at 253.7 nm. These solvents should not be present anywhere near the location where this determination is being performed.
3. Chlorine and oxides of nitrogen may interfere by absorbing at 253.7 nm. If any of these interferences are suspected, test samples should be analyzed without the reducing agent to confirm 0 absorbance.
4. Various elements and compounds may cause chemical interferences which yield a low bias. These include Au, Pd, Pt and Te at levels over 0.03, 0.15, 0.01 and 0.04 mg/L, respectively. Copper also interferes at levels over 10 mg/L. Arsenic, Se, Sb, Bi and sulphides cause similar interferences as well. Since the concentration of these elements and compounds in fish tissue is normally low, such interferences usually do not affect the accuracy of the analyses.

Sample Preparation and Storage

1. Fish muscle should be submitted to the laboratory as prepared filets. See Appendix I of this method for fileting procedures.
2. Plastic bags (Whirl-Pak™, or equivalent) should be used for storing the fish tissue. The air space in the bag should be minimized to avoid losses in the water content of the sample.
3. Fish samples may be transported chilled (on ice) if only short distances are involved, but preferably samples should be frozen (≤-20°C) prior to shipment to the laboratory. Freezing is the only suitable method of sample preservation.
4. Subsample preparation and subsampling procedure: See Appendix II of this method.

Apparatus

1. Analytical balance, readable to 0.1 mg (Mettler AE 163, or equivalent).
2. Digestion tubes: Folin-Wu, graduated at 25 and 50 mL (Corning 7900-25, Kimble 47125-50, or equivalent). 40 tubes are required.
3. Block digester with a 40-tube capacity, capable of variable temperature control to 250°C (Technicon BD-40, or equivalent).
4. Micropipettor, adjustable, calibrated, capable of accurate delivery of 0.2 - 1.0 mL.
5. Vortex mixer (Scientific Industries Inc. Vortex-Genie, or equivalent).
6. AutoAnalyzer system consisting of the following (see Figure 1, manifold diagram):
   (a) Sampler.
   (b) Analytical module.
   (c) Proportioning pump.
7. Gas/liquid separator (Figure 2).
8. Mercury monitor (LDC/Milton Roy uvMonitor™ 1255, or equivalent), equipped with dual channel, long path length absorbance cell (295 mm long by 7 mm diameter), and configured to measure absorbance at 253.7 nm. Nominal absorbance range setting on the uvMonitor™ 1255 is 0.16 units.
9. Heating tape (6 feet by 1 inch) and variable transformer (0 - 120 V output). The tape is wrapped around the absorbance cell to maintain a temperature of 50 - 60°C; a transformer output of 20% is sufficient to provide this temperature.
10. Chart recorder, 10 mV full scale. Chart speed should be set to 0.5 cm/min.

Reagents

1. Deionized, distilled water (DDW)
2. Digestion acid (HNO_3:H_2SO_4 -- 1:2 by volume): Add, with continuous mixing, 1 L concentrated reagent grade sulphuric acid (specific gravity 1.84) into 500 mL concentrated reagent grade nitric acid (specific gravity 1.42). Allow to cool to room temperature before using.

   CAUTION: CONCENTRATED H_2SO_4 AND HNO_3 ACIDS WILL CAUSE SERIOUS BURNS UPON CONTACT WITH SKIN!

3. Wash water (20% H_2SO_4): Carefully add 400 mL concentrated reagent grade H_2SO_4 into approximately 1500 mL DDW. Dilute to 2 L with DDW when cool and store in a glass reagent bottle.
4. Reducing reagent (4% $SnCl_2 \cdot 2H_2O$, 4% HCl, 2% $(NH_2OH)_2 \cdot H_2SO_4$, 1% NaCl): Dissolve 40 g of stannous chloride in 40 mL concentrated reagent grade hydrochloric acid. Dilute to approximately 200 mL with DDW. Into this solution, dissolve 20 g of reagent grade hydroxylamine sulphate and 10 g of reagent grade sodium chloride, and dilute to 1 L with DDW in a volumetric flask.
5. Mercury absorbing solution: Combine equal volumes of 10% H_2SO_4 and 0.5% $KMnO_4$.
6. Compressed nitrogen: Nitrogen that has been confirmed to be free of mercury and organic compounds. The nitrogen source should be fitted with regulators to provide a nominal flow rate of 50 mL/min.
7. Preservative (3% $K_2Cr_2O_7$ - 50% HNO_3): Add 30 g potassium dichromate to 500 mL of reagent grade concentrated nitric acid and dilute to 1 L with DDW. Confirm that this solution yields a negligible blank. The preservative is used at a strength of 16 mL preservative per litre preserved solution.
8. Stock Mercury Standard Solution (1000 mg/L): Purchase a 1000 mg/L atomic spectroscopy standard or dissolve 0.6768 g of reagent grade $HgCl_2$ in 8 mL of preservative and approximately 400 mL of DDW, and dilute to 500 mL in a volumetric flask.
9. Intermediate Mercury Standard Solution (10 mg/L): Dilute a 5.0 mL aliquot of Stock Mercury Standard Solution and 8 mL of preservative to 500 mL in a volumetric flask.
10. Working Mercury Standard Solution (250 µg/L): Dilute a 25.0 mL aliquot of Intermediate Mercury Standard Solution and 16 mL of preservative to 1 L in a volumetric flask.
11. Quality Control Solutions:

(a) Stock Mercury QC Solution (500 mg/L Hg in 1*N* H_2SO_4): Dissolve 0.4197 g of phenyl mercuric acetate (MW = 336.74 g/mol) in 14 mL of reagent grade sulphuric

acid, 8 mL of preservative, and approximately 400 mL of DDW in a 500 mL volumetric flask. Dilute to volume.

(b) Intermediate Mercury QC Solution (10 mg/L): Dilute a 10.0 mL aliquot of Stock Mercury QC Solution and 8 mL of preservative to 500 mL in a volumetric flask.

(c) Working Mercury QC Solution (250 µg/L): Dilute a 25.0 mL aliquot of Intermediate Mercury QC Solution and 16 mL of preservative to 1 L with DDW in a volumetric flask.

12. Conditioning Solution (0.1 mg/L Hg): Dilute a 5 mL aliquot of Intermediate Mercury Standard and 8 mL of preservative to 500 mL with DDW in a volumetric flask.

Procedure

A. Preparation of Standards

1. Add the following volumes of 250 µg/L Working Mercury Standard solution to the first 11 digestion tubes:

Volume of 250 µg/L Hg Standard (mL)	Amount of Hg Dispensed (µg)
0(blank)	0(blank)
0.2	0.05
0.4	0.10
0.6	0.15
0.8	0.20
1.0	0.25
1.2	0.30
1.4	0.35
1.6	0.40
1.8	0.45
2.0	0.50

B. Preparation of QC Materials

2. QC Solutions: Dispense the following volumes of 250 µg/L Working Mercury QC solution into the next 3 digestion tubes:

Volume of 250 µg/L Hg QC Solution (mL)	Amount of Hg Dispensed (µg)
1.5	0.375
0.5	0.125
0	0

3. QC Tissues: Dispense in-house and/or certified reference fish tissue into the next 2 to 4 digestion tubes. Weights of dried or semi-dried materials should not exceed 0.5 g wet weight equivalent (assume 80% moisture content in wet weight).
4. Duplicates: Dispense two duplicate samples selected at random from the analytical samples in the next 2 digestion tubes, according to the instructions for dispensing samples given below.

C. Dispensing Fish Samples

5. Dispense 0.3 - 0.5 g (ideally 0.4 g) of fish tissue, directly into the remaining digestion tubes, weighing the tissue accurately to the nearest 0.1 mg. Maintain the tissue temperature as near as possible to 0°C during pre-weighing manipulations, in order to avoid moisture evaporation. Also, when dispensing the tissue, ensure that it is set at the bottom of the tube. If some sample does stick to the tube wall, drop it to the bottom by gently tapping the tube on a firm, cushioned surface. Do not rinse tissue down with water, as the presence of more than 2 mL of water can cause bumping during digestion.

D. Sample Digestion

6. Add 7.5 mL digestion acid to all tubes.
7. Dissolve tissues by placing all tubes in the digestion block, pre-heated to 60°C. All tissues should dissolve within one-half hour of heating. Swirl to mix any layers that may form.
8. Increase temperature of the digestion block to 95°C and allow to stabilize (0.25 - 0.50 hr). Set the automatic temperature program for 1.5 hr @ 180°C followed by 5.5 hr @ 250°C. Engage the program and allow the digestion to proceed overnight.
   CAUTION: DIGESTION SHOULD BE PERFORMED IN A FUMEHOOD.
9. By morning, all digests will have cooled to room temperature. Remove them from the digestion block.
10. While mixing on a vortex mixer, slowly add about 15 mL DDW to all the digests.

CAUTION: AS THE DIGESTS ARE ESSENTIALLY CONCENTRATED SULPHURIC ACID, ADDING WATER TO THEM PRODUCES SUBSTANTIAL HEAT WHICH MAY CAUSE SPATTERING!

Diluting the digests with vigorous mixing, and adding enough water to absorb much of the heat evolved, prevents boiling and bumping losses. Dilute to about 24 mL, mix, and allow to cool. Once cool, dilute to the 25 mL mark, and mix again.

E. Mercury Analysis

11. Set up the analytical system as depicted in Figure 1. Condition the system by passing a portion of 0.1 mg/L conditioning solution.
12. After a stable baseline is obtained, analyze the digests in the following sequence: Standards, QC solutions, QC tissues, Duplicates, wash, first half of samples, wash, second half of samples, wash, standards. Standards are analyzed in order from high to low.

Calculations and Method of Reporting

Construct a calibration curve of peak height versus amount of mercury from the standards. Use a second order least squares regression to fit the data to the formula

$$h = ax^2 + bx + c$$

where h is peak height in mm, x is amount of mercury in µg, and a, b, and c are the fitted second, first, and zero order coefficients, respectively. Graph the fitted curve to confirm its validity. A typical calibration curve is shown in Figure 3.

From the fitted second order curve, calculate the amount of mercury in the digests by the formula

$$x = \frac{-b + \sqrt{b^2 + 4a(h-c)}}{2a}$$

Express the concentration of mercury in the tissues as mg/kg, computed by the formula

$$C = \frac{x}{w}$$

where C is the concentration of mercury and w is the weight, in grams, of tissue digested. Report the results when the QC data are in control.

Precision and Accuracy

1. Within-run Precision (derived from the analysis of duplicates).

   (a) Homogenized Samples:

S_w (mg/kg)	Concentration Range (mg/kg)	n
0.0031	< 0.25	6
0.0061	0.25 - 0.50	15
0.0095	0.50 - 1.00	14

   (b) Snipped Samples (derived from snips taken from adjacent tissues, as described in Appendix II.A):

S_w (mg/kg)	Concentration Range (mg/kg)	n
0.0060	< 0.25	32
0.0082	0.25 - 0.50	11

2. Between-run Precision

   (a) Aqueous Standards:

S (µg)	Mercury Level (µg)	n
0.0053	0.125	37
0.0076	0.375	38

(b) Reference Material (freeze-dried, defatted fish muscle):

S (mg/kg)	Concentration (mg/kg)	n
0.1829	3.795	36
0.0814	1.416	35

3. Accuracy

(a) Certified Reference Material DORM-1 (Dogfish Muscle from National Research Council of Canada):

Certified Concentration ± Std. Dev. (mg/kg)	Observed Concentration ± Std. Dev. (mg/kg)	n	Recovery (%)
0.798 ± 0.074	0.859 ± 0.061	34	108

(b) Inter-Laboratory Comparison: This method was used to analyze samples from the Mercury Quality Assurance Program conducted by Fisheries and Oceans Canada's Freshwater Institute, Winnipeg, Manitoba. Over one year (sample numbers 220 - 231), the observed concentration of 11 of the 12 samples distributed was within one standard deviation of the corrected overall mean, while the twelfth was within two standard deviations. The observed average recovery on these samples, representing the concentration range 0.1 - 1.0 mg/kg, was 103%. None of the data generated by this method was rejected as an outlier.

References

1. Alberta Environmental Centre. "Total Mercury in Fish Tissue (Manual Persulphate Digestion, UV Mercury Monitor Method)". *Methods Manual for Chemical Analysis of Water and Wastes*. AECV87-M1, 1987.
2. Ontario Ministry of the Environment, Laboratory Services Branch. "The Determination of Mercury in Fish by AAS". Method HGBIO-E3057A.1, March 6, 1989.

3. Environment Canada. "Mercury in Fish (Cold Vapour Atomic Absorption)". *Analytical Methods Manual Update*. Inland Waters Directorate, Water Quality Branch, Ottawa, Ontario, 1981.

4. United States Environmental Protection Agency, Environmental Monitoring and Support Laboratory. "Mercury, Method 245.1 (Manual Cold Vapour Technique)." *Methods for Chemical Analysis of Water and Wastes, EPA-600/4-82-055*. December, 1982.

This Method has been written by D. Lucyk based upon the development work completed by D. Lucyk, verified by S. Wu, and reviewed by F.P. Dieken. Appendix I has also been reviewed by K. Smiley and J. Moore. This method of analysis was implemented in the Water Analysis Laboratory in December, 1991.

Figure 1 -- Mercury in Fish Analytical Manifold

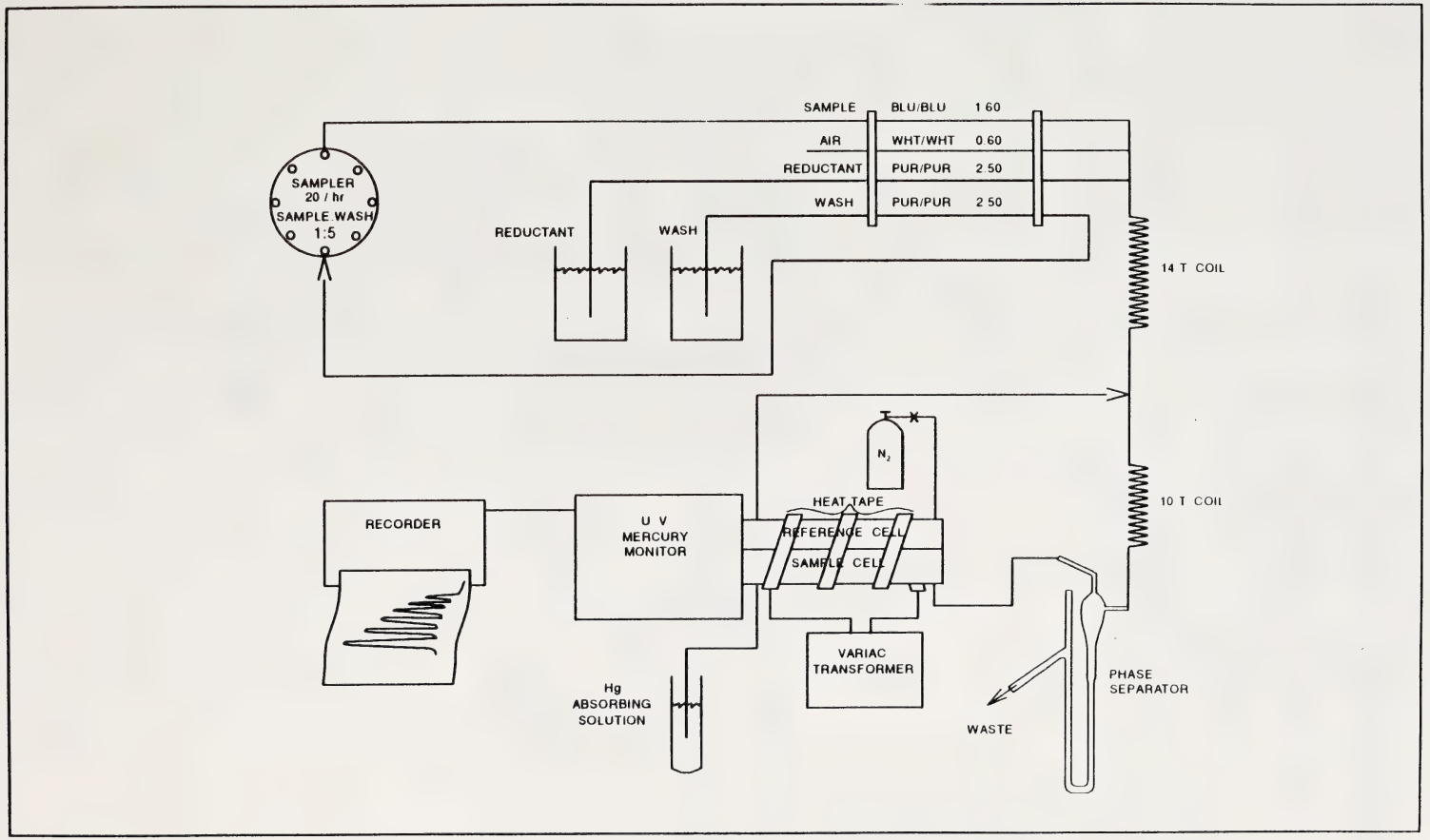


Figure 2 -- Mercury System Gas/Liquid Separator

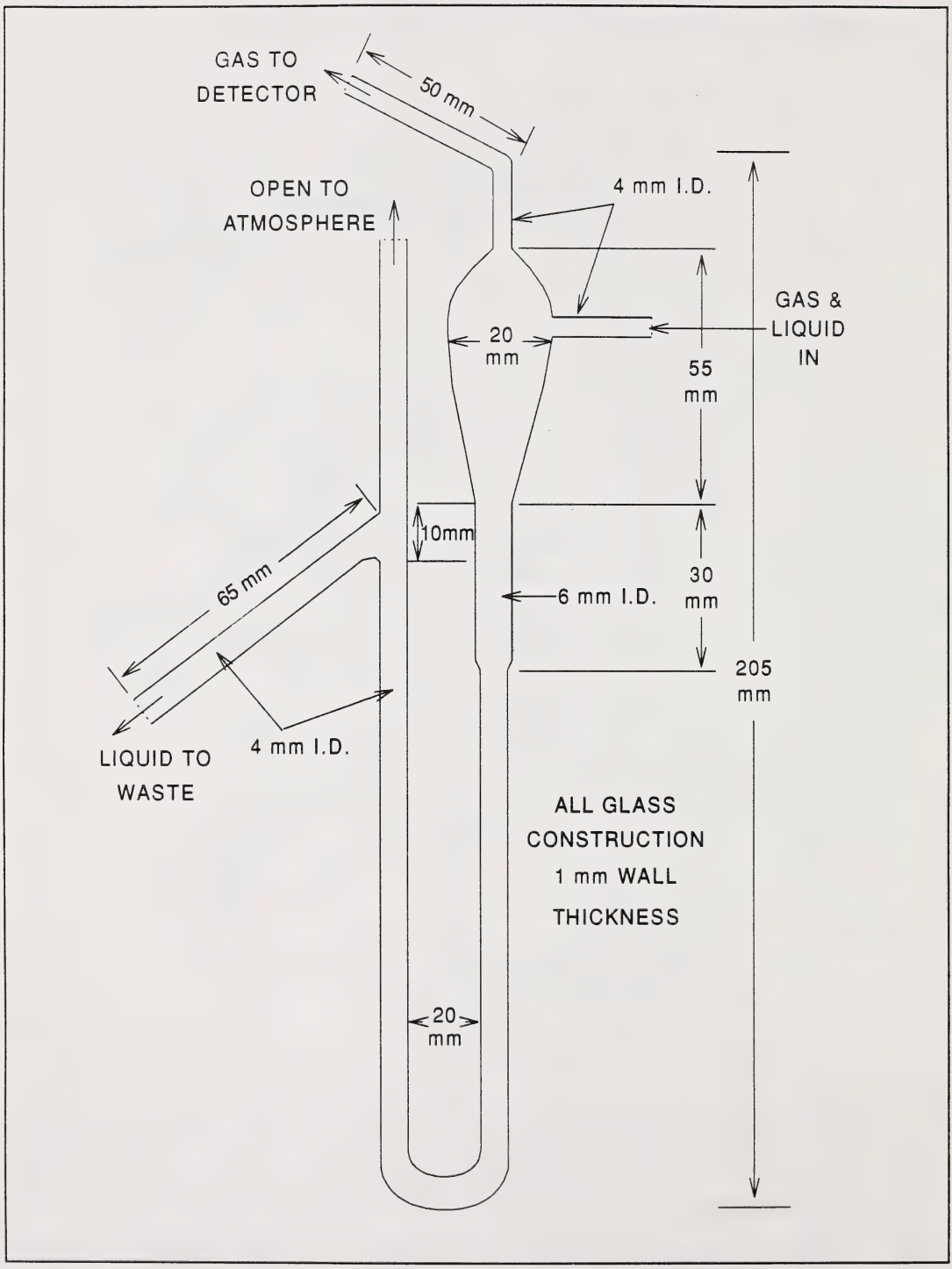
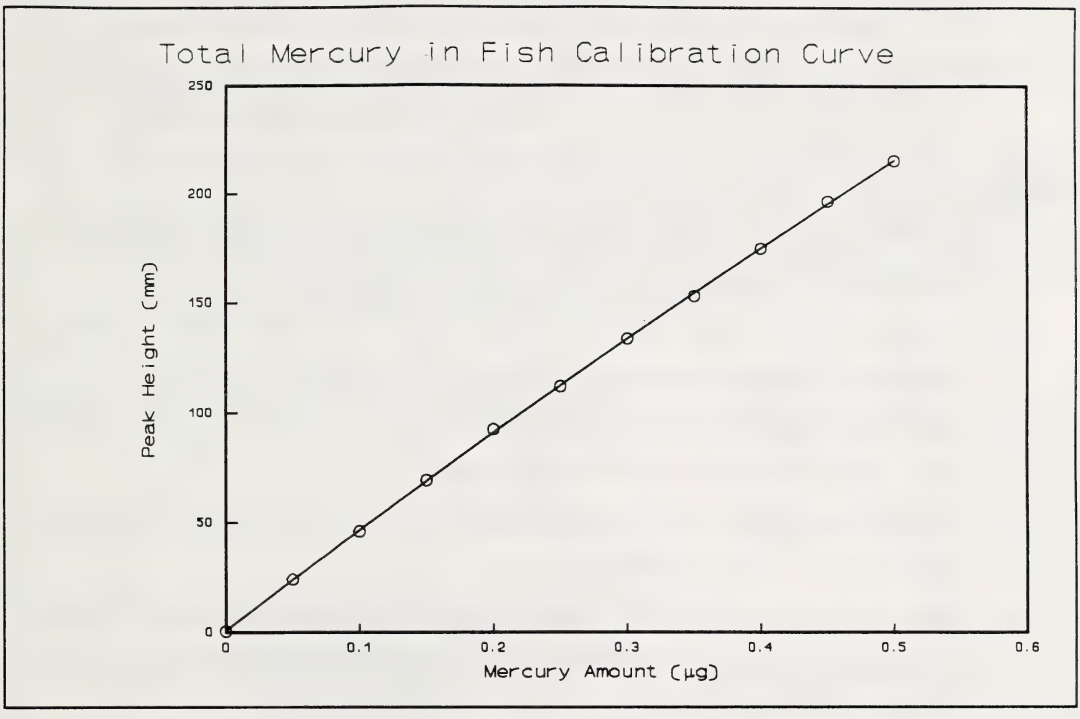


Figure 3 -- Typical Calibration Curve



APPENDIX I

SAMPLING FISH MUSCLE TISSUE FOR MERCURY ANALYSIS

Materials

1. Stainless steel knives and/or scalpels of surgical quality. Specific sizes and types of these utensils will depend on the fish sizes and species dissected.
2. Cutting table that can be cleaned and rinsed easily. The cutting surface should be composed of linear polyethylene or other suitable non-metallic material.
3. Disinfectant (Savlon™, Hibitaine™, or equivalent).
4. Plastic sample bags (Whirl-Pak™, or equivalent).
5. Fish measuring board, with 1 m ruler having 1 mm sub-divisions imbedded in the surface.
6. Water for rinsing utensils and cutting surface before sampling each fish. The water supply must be metal-free: in the laboratory, use DDW; in the field, use a treated supply of known (high) quality.
7. Toploading balance fitted with large pan accessory, readable to 0.1 g, with range appropriate for specimens being tested (Mettler PK 36, or equivalent).
8. Freezer, capable of maintaining a temperature of -20°C.
9. Refrigerator, capable of maintaining a temperature of 4°C.

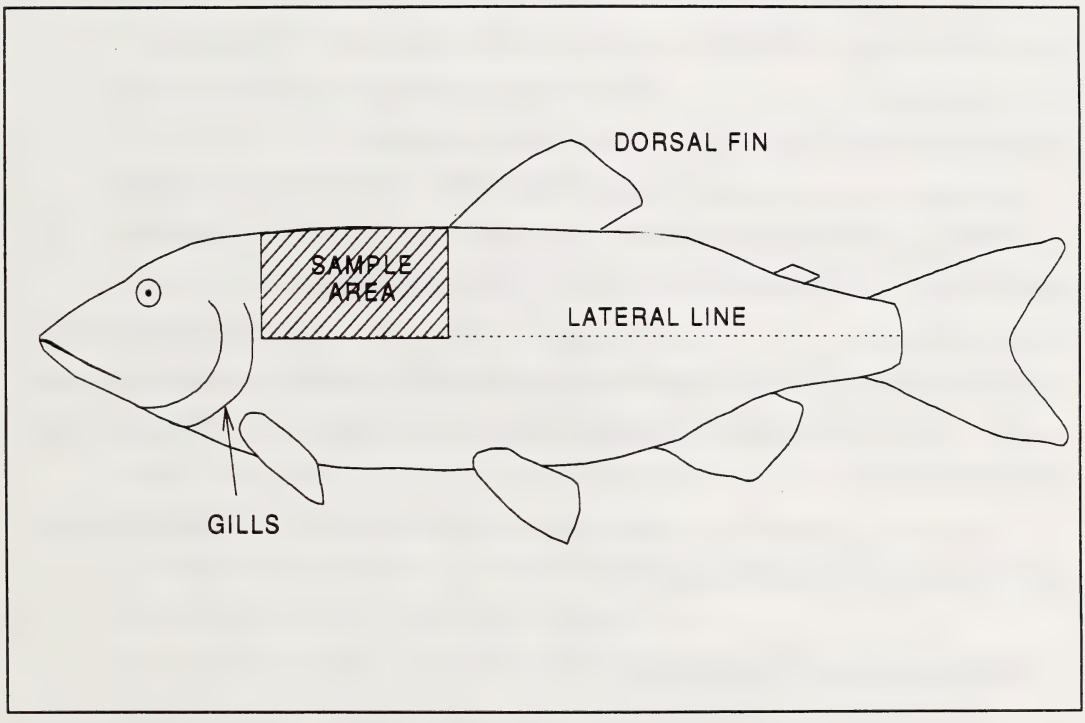
Procedure

1. Soak dissecting utensils in Savlon™ solution overnight, and rinse well before use. Wash down the cutting surface and utensils with Hibitaine™ solution and rinse well with water between samples.
2. Record fish lengths and weights at time of capture. Fish should be fileted within two hours of collection or frozen whole as soon as possible and stored at -20°C. Frozen fish are thawed (4°C) before fileting and lengths and weights are recorded; dissections should be completed as soon as possible after thawing.
3. The tissue sample should consist of skinless epaxial (ie. above the lateral line) muscle taken from the left side of the fish, from an area immediately behind

the gills to just before the dorsal fin (see Figure I). For some fish species, the dorsal fin does not provide a good landmark, being too near the gills (eg. yellow perch) or too far from the gills (eg. northern pike); in such cases, use the mid-body point as the posterior sample location.

4. If the tissue sample is not analyzed immediately, it is to be stored in a plastic bag at -20°C. Before freezing, the bag should be wrapped tightly around the filet and excess air should be excluded from the bag. The bag should be clearly labelled with a unique, individually trackable sample number.

Figure I -- Sample Area for Mercury Analysis of Fish



APPENDIX II

SUBSAMPLING FISH MUSCLE TISSUE FOR MERCURY ANALYSIS

Fish muscle tissue received in the lab generally consists of a skinless filet of epaxial muscle taken from the front, left side of the animal (see Appendix I). This filet may be homogenized before subsampling or may be left intact and subsampled by excising a piece of tissue, hereafter referred to as "snipping".

The bulk of routine samples processed in the Water Analysis Lab (WAL) are subsampled by snipping. Snipping is a rapid and inexpensive method of subsampling, which does not subject the sample to a high risk of contamination or moisture loss. It is useful for processing samples as small as 1 g or less. Its major drawback is that it contributes to sampling error, thereby decreasing the overall method precision.

Subsampling of homogenates is usually carried out at WAL on samples which are received homogenized or on samples of a non-routine nature. The main advantage of homogenization is that sampling error due to varying concentrations within the tissue is effectively eliminated. However, homogenization is time and labour intensive, and generally requires large samples (>15 g). Since this is a very intrusive and harsh procedure, care must be taken to limit contamination and moisture evaporation, as both of these will impart a bias to the data.

In either case, preparation for subsampling should be performed on soft-frozen (i.e. at or near 0°C) tissue to minimize moisture loss.

A) SUBSAMPLING BY SNIPPING

A snip of tissue may be taken from a muscle filet by cutting out an appropriately sized subsample with a scalpel. The snip should be taken from the middle of the sample's thickness to avoid surface tissue which may have been exposed to contamination or may have dried out during storage.

Apparatus

1. Cutting board or other suitable surface, of non-metallic construction.
2. Waxed paper and/or plastic wrap.
3. Scalpels: #4 handle with #22 blade and #3 handle with #11 blade.
4. Tweezers.
5. Toploading balance, with 0.01 gram readability.
6. Analytical balance, readable to 0.1 mg (Mettler AE 163, or equivalent).

Procedure

1. Clean the cutting surface and cover with a sheet of waxed paper (or plastic wrap). Use a fresh sheet of waxed paper for each filet. Scalpel blades should be changed daily and wiped with clean tissues between filets.
2. Lay the filet flat on the cutting surface and slice it lengthwise through most of its thickness, leaving the two halves attached (Figure II.a).
3. Make two angular, lengthwise cuts along one of the exposed surfaces to form a "v" (Figure II.b). Make two more cuts near the ends of the filet, perpendicular to the angular incisions.
4. With a pair of tweezers, lift out the v-shaped snip (Figure II.c). Confirm that the snip is of a weight appropriate for the analysis (0.4 g for total mercury; 1 g for organic mercury) by weighing on a toploading balance. Use waxed paper to shield the sample from the metal pan.
5. Transfer the snip directly into the digestion/extraction tube -- weighing it accurately to the nearest 0.1 mg -- and proceed with analysis.
6. If a duplicate is required, repeat steps 3 through 5 for the other surface exposed in step 2.

B) SUBSAMPLING HOMOGENATES

Apparatus

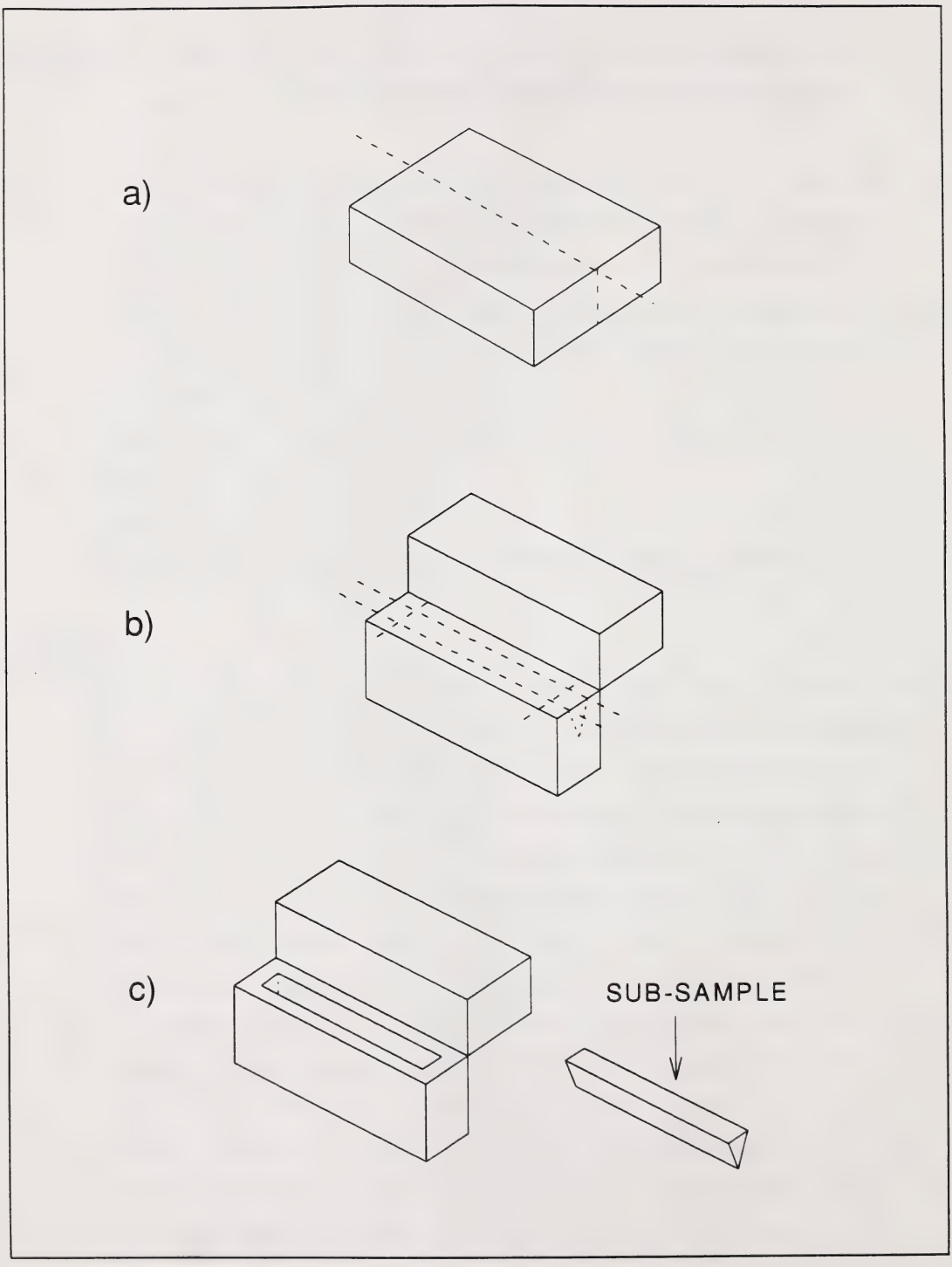
1. Mechanical homogenizer (Virtis 45, The Virtis Company, Gardiner NY, or equivalent).
2. Homogenization vessels: stainless steel or sturdy glass (CAUTION: The Virtis homogenizer mentioned above has soft-metal blades which allow using large, rounded glass centrifuge bottles as homogenizing vessels, without much risk of breakage; this allows the operator to observe the progress of homogenization. Nevertheless, appropriate safety precautions should always be observed. WEAR SUITABLE EYE AND HAND PROTECTION!
3. Cutting board or other suitable surface of non-metallic construction.
4. Waxed paper and/or plastic wrap.
5. Scalpel: #4 handle with #22 blade.
6. Analytical balance, readable to 0.1 mg (Mettler AE 163, or equivalent).

Procedure

1. Clean the entire apparatus before processing each sample. Wipe the cutting surface and lay down a fresh sheet of waxed paper. The scalpel blade should be changed daily and wiped with a clean tissue between filets. The homogenizer shaft and blades should be wiped thoroughly with clean tissues. Homogenizing vessels are best cleaned with cool water and a bottle brush, then rinsed with DDW and air- or tissue-dried; glass vessels can be rinsed with nitric acid prior to the DDW rinse.
2. Place the filet on the cutting surface and cut it into pieces of a workable size. Load all pieces into a digestion vessel and attach the vessel to the homogenizer. Homogenize at a medium-low setting. If tissue adheres to the vessel walls, turn off the homogenizer and push the tissue to the bottom of the vessel with a clean spatula. Repeat homogenization until a smooth paste is obtained.
3. Transfer a subsample of appropriate weight (0.4 g for total mercury; 1 g for organic mercury) directly into the digestion/extraction tube, using a laboratory spatula or other suitable implement. Record the weight accurately to the nearest 0.1 mg.

4. If the remaining tissue is to be stored, it may be returned to its original plastic sample bag or transferred to some other suitable container for freezing. If this residual homogenate is re-sampled at a later date, mix it thoroughly as the various phases (water, tissue, fat) may separate during storage.

Figure II -- Subsampling by Snipping



Appendix D

Analytical Method: Organic Mercury in Fish Tissue

ORGANIC MERCURY IN FISH TISSUE

(Solvent Extraction, Acid Digestion, Cold-Vapour Atomic Absorption Method)

Introduction

This method consists of extracting organic mercury from fish tissue into an organic solvent followed by analysis of the extract for total mercury. The extraction method is an adoption of a method developed by the Canadian Department of Fisheries and Oceans, Inspection Services Branch, Winnipeg, Manitoba. Total mercury in the extract is determined using the method "Total Mercury in Fish Tissue" included in this manual.

Although the extraction is designed to extract methyl mercury from fish tissue, it is not specific for this chemical species. If present, other protein-bound organo-mercurials will be extracted as well. For this reason, the method is termed "organic" mercury in fish rather than "methyl" mercury.

Summary of Method

Protein-bound organic mercury compounds are released from fish tissue with an acidic aqueous mixture of cupric sulphate and sodium bromide and are extracted as bromide complexes into methylene chloride. A portion of the methylene chloride extract is analyzed for total mercury.

Scope

This method is applicable to the analysis of organic mercury in fish muscle tissue and may be applicable to whole fish, fish organs or other animal tissues. Based on a practical sample size of 1 g, the analytical range is 0.02 - 1.25 mg/kg mercury on a wet weight basis.

Interferences

1. Ultra-violet light will decompose the CH_3Hg^+ ion, therefore exposure of sample and standard solutions to light should be minimized and exposure to direct sunlight must be avoided.

2. Precautions regarding interferences in the analysis for total mercury should be noted (see "Total Mercury in Fish Tissue" in this manual).

Sampling and Storage

1. Fish muscle should be submitted to the laboratory as prepared filets. See Appendix I of "Total Mercury in Fish Tissue" in this manual for fileting procedures.
2. Plastic bags (Whirl-Pak™, or equivalent) should be used for storing fish tissue. The air space in the bag should be minimized to avoid losses in the water content of the sample.
3. Fish samples may be transported chilled (on ice) if only short distances are involved (less than 1 day), but preferably samples should be frozen (≤-20°C) prior to shipment to the laboratory. Freezing is the only suitable method of sample preservation.
4. See Appendix II of "Total Mercury in Fish Tissue" in this manual for preparation and subsampling procedures.

Apparatus

1. Centrifuge tubes, glass, round bottom (Kimble 45212-35, Kimble 45207-40, or equivalent). 40 tubes required.
2. Centrifuge capable of speeds of at least 3000 rpm.
3. Tissue homogenizer (Polytron, or equivalent).
4. Vortex mixer (Scientific Industries Inc. Vortex-Genie, or equivalent).
5. Micropipettor, adjustable, calibrated, capable of accurately delivering 0.010 - 0.100 mL.
6. Micropipettor or gas-tight syringe, capable of delivering 1.0 mL accurately and reproducibly.
7. Digestion Tubes: Folin-Wu, calibrated and graduated at 25 and 50 mL (Corning 7900-25, Kimble 47125-50, or equivalent). 40 tubes required.
8. Apparatus listed in "Total Mercury in Fish Tissue" method in this manual.

Reagents

1. Deionized, distilled water (DDW)
2. 4*N* Sulphuric acid: Slowly add, with mixing, 222 mL of concentrated reagent grade H_2SO_4 (specific gravity 1.84) to approximately 1500 mL DDW. Allow to cool and dilute to 2 L.

   CAUTION: CONCENTRATED H_2SO_4 WILL CAUSE SERIOUS BURNS.
3. Cupric Sulphate solution (2.5% in 4*N* H_2SO_4): In a 1 L volumetric flask, containing about 750 mL 4*N* H_2SO_4, dissolve 25 g $CuSO_4 \cdot H_2O$. Dilute to volume with 4*N* H_2SO_4. Store in a brown glass bottle.
4. Sodium bromide solution (30% in 4*N* H_2SO_4): Dispense 75 g NaBr into a 250 mL volumetric flask. Dissolve and dilute to volume with 4*N* H_2SO_4. Stable for 1 week.
5. Methylene chloride (pesticide grade or better).
6. Methanol (pesticide grade or better).
7. Stock Standard Mercury Solution (1250 mg/L): Dissolve 0.1565 g CH_3HgCl (methyl mercuric chloride) in methanol and dilute to 100 mL with methanol in a volumetric flask. Store in the dark at 4°C.
8. Working Mercury Standard Solution (12.50 mg/L): Dilute a room temperature 1 mL aliquot of Stock Mercury Standard Solution to 100 mL in a volumetric flask with DDW. Store in the dark at 4°C.
9. Quality Control Solutions:
   a. Stock Mercury QC solution (1250 mg/L): Dissolve 0.1565 g of CH_3HgCl, obtained from a supplier other than the chemical used to prepare the Standard solution, in methanol and dilute to 100 mL with methanol in a volumetric flask. Store in the dark at 4°C.
   b. Working Mercury QC solution (12.50 mg/L): Dilute at room temperature 1 mL aliquot of Stock Mercury QC solution to 100 mL with DDW in a volumetric flask. Store in the dark at 4°C.
10. Prepare reagents listed in "Total Mercury in Fish Tissue" method in this manual, except standard and QC solutions.

Procedure

A. Preparation of Standards

1. Dispense the following volumes of 12.50 mg/L Working Mercury Standard solution into the first 11 centrifuge tubes:

Volume of 12.50 mg/L Hg Standard (mL)	Amount of Hg Dispensed (ug)
0	0
0.010	0.125
0.020	0.250
0.030	0.375
0.040	0.500
0.050	0.625
0.060	0.750
0.070	0.875
0.080	1.000
0.090	1.125
0.100	1.250

B. QC Materials

2. QC Solutions: Dispense the following volumes of 12.50 mg/L Working Mercury QC solution into the next 3 centrifuge tubes:

Volume of 12.50 mg/L Hg QC Solution (mL)	Amount of Hg Dispensed (ug)
0.075	0.9375
0.025	0.3125
0	0

3. QC Tissues: Dispense in-house and/or certified reference fish tissue into the next 2 to 4 digestion tubes. Weights of dried or semi-dried materials should not exceed 1 g wet weight equivalent (assume 80% moisture content in wet weight).

4. Duplicates: Dispense two duplicate samples selected at random from the analytical samples into the next 2 digestion tubes, according to the instructions for dispensing samples given in the next procedural step.

C. Dispensing Fish Samples

5. Dispense 1 g samples of fish tissue into the remaining centrifuge tubes. Maintain the tissue temperature as near as possible to 0°C during pre-weighing manipulations, in order to avoid moisture evaporation.

D. Solvent Extraction

6. Dispense 7.5 mL copper sulphate solution and 5.0 mL sodium bromide solution into each tube.
7. Homogenize for one minute (medium speed setting on Polytron).
8. Add 5.0 mL methylene chloride, cap the tubes, and vortex for five 5-second intervals at medium speed setting.
9. Aspirate off most of the aqueous layer and centrifuge at 2500 - 3000 rpm for 5 minutes.
10. Transfer 1.0 mL aliquots of the methylene chloride extracts into digestion tubes, taking care to dispense the extract below the 25-mL mark on the tubes.

E. Mercury Analysis

11. Add 7.5 mL digestion acid to all tubes.
12. Analyze extracts according to the method "Total Mercury in Fish Tissues" in this manual, starting at procedural step 8. Nominal absorbance range setting on the uvMonitor™ 1255 is 0.08 units.

Calculations and Method of Reporting

Construct a calibration curve of peak height versus amount of mercury from the standards. Use a second order least squares regression to fit the data to the formula

$$h = ax^2 + bx + c$$

where h is peak height in mm, x is amount of mercury in µg, and a, b, and c are the fitted second, first, and zero order coefficients, respectively. Graph the fitted curve to confirm its validity. A typical calibration curve is shown in Figure 1.

Calculate the amount of mercury in the extracts from the fitted second order curve using the formula

$$x = \frac{-b + \sqrt{b^2 + 4a(h-c)}}{2a}$$

and express the concentration of mercury in the tissues as mg/kg, computed by the formula

$$C = \frac{x}{w}$$

where C is the concentration of mercury and w is the weight, in grams, of tissue extracted.

Report the results when the QC solutions and the reference materials are in control.

Precision and Accuracy

1. Within-run Precision derived from the analysis of duplicates.

   (a) Homogenized Samples:

S_w (mg/kg)	Concentration Range (mg/kg)	n
0.0069	< 0.25	4
0.0089	0.25 - 0.50	5
0.0137	0.50 - 1.00	4

   (b) Snipped Samples (derived from snips taken from adjacent tissues, as described in Appendix II.A of "Total Mercury in Fish Tissue" in this manual):

S_w (mg/kg)	Concentration Range (mg/kg)	n
0.0054	< 0.25	10
0.0085	0.25 - 0.50	1

2. Between-run Precision

   (a) Aqueous Standards:

S (µg)	Mercury Level (µg)	n
0.0161	0.3125	10
0.0321	0.9375	10

(b) Reference Material (freeze-dried, defatted fish muscle):

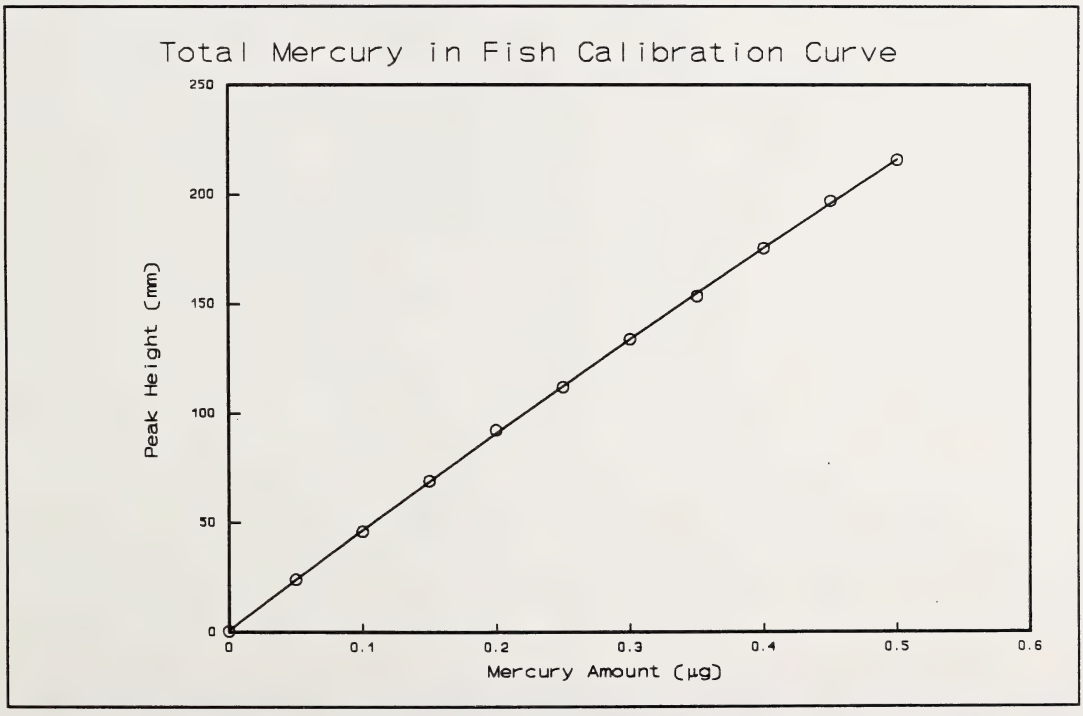
S (mg/kg)	Concentration (mg/kg)	n
0.0672	3.272	11
0.0432	1.184	10

3. Accuracy

Based on the analysis of National Research Council of Canada certified reference material DORM-1 (Dogfish Muscle):

Certified Concentration ± Std. Dev. (mg/kg)	Observed Concentration ± Std. Dev. (mg/kg)	n	Average Recovery (%)
0.731 ± 0.060	0.748 ± 0.043	10	102

Figure 1 -- Typical Calibration Curve



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This method has been written by D. Lucyk based upon the development work completed by D. Lucyk, verified by S. Wu and reviewed by F.P. Dieken. This method was implemented in the Water Analysis Laboratory in March, 1992.